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OOGENESIS IN PHILOSAMIA CYNTHIA

PAULINE H. DEDERER

From the Zoological Laboratory, Columbia University

SIXTY-FOUR FIGURES (SIX PLATES)

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INTRODUCTION

From the standpoint of sex production the Lepidoptera are of especial interest as compared with other insects, because the experimental evidence of Doncaster ('06, '08) and Raynor ('06), Punnett and Bateson ('08) seems to demand the assumption that there are two kinds of eggs in the moth. The absence of visible dimorphism in the spermatozoa of the Lepidoptera also lends probability to this hypothesis. In other groups of insects studied, the spermatozoa are often dimorphic. The eggs have been assumed to be all alike, and this condition has

been demonstrated by Morrill ('09) for certain coreid Hemiptera, by Morgan ('09) for phylloxerans, by Stevens ('06 a, '09) and von Baehr ('08, '09) for aphids.

In studying the history of the male germ cells in the moth *Philosamia cynthia* (Dederer '07), the spermatocytes were found to contain exactly similar groups of chromosomes. The same facts had been determined by Stevens ('06 b) and Cook ('10) for various other Saturniidae. Doncaster ('12) believes that in *Pieris brassicae* there is no dimorphism, either in the male or female germ cells. Recently, however, Seiler ('13) has described two kinds of eggs in a lepidopteran. These two papers will be discussed later. The present work was undertaken with special reference to the question of dimorphism in the eggs. I wish to express my indebtedness to Prof. E. B. Wilson for valuable advice and criticism during the course of the investigation.

MATERIAL AND TECHNIQUE

Carnoy's aceto-alcohol-chloroform mixture, saturated with sublimate, was used almost exclusively, since it readily penetrated the tough chorion of the eggs, which were left in the fluid from two to four hours. Flemming's and Bouin's fluids were used, but proved very unsatisfactory. The eggs were then transferred to iodized 95 per cent alcohol, and the chorion was removed with needles. After dehydration in absolute alcohol, the eggs were placed in a mixture of alcohol and chloroform followed by pure chloroform for ten minutes. Immersion in melted paraffine for fifteen minutes was sufficient for perfect infiltration. The stains used were iron hematoxylin, and in a few cases safranin.

The maturation spindle remains in first metaphase until the entrance of the sperm. The following tabulation gives roughly the maturation stages at different intervals after the eggs are laid:

Eggs just laid to $\frac{1}{2}$ hour laid	first anaphase
Eggs laid 1 to $1\frac{1}{2}$ hours	second metaphase
Eggs laid $1\frac{3}{4}$ hours	second telophase
Eggs laid 2 to $2\frac{3}{4}$ hours	fusion of pronuclei

In the study of the early stages of oogenesis, both caterpillars and pupae were used. The ovaries in the early pupal stage lie enveloped in the fat bodies just beneath the dorsal wall of the abdomen in the fifth segment. Upon removing the wall, the ovaries were located and transferred immediately to the fixing fluid. Flemming's, Bouin's and Carnoy's fluids were used, the latter being the only one good for all stages. The stains employed were safranin and iron hematoxylin.

MATURATION DIVISIONS

1. OBSERVATIONS

a. Chromosomes in the embryo

The number of chromosomes in the spermatogonia is 26 (Dederer '07), all rounded bodies, approximately equal in size. For determining the somatic number, sections were made of eggs several hours after fertilization. Ten counts from three different lots of eggs showed clearly 26 chromosomes. In polar view of metaphase (fig. 1) they appear as slightly elongated, often bipartite, bodies of comparatively slight difference in size. Owing to their similarity it is impossible to attempt any arrangement of the chromosomes in pairs, as has been done in some animals.

b. First maturation division

The mature eggs are oval bodies about 1.5 mm. long, each invested in a tough white chorion, which is flatter and broader at the animal pole. The flattened area appears to be due to the contact of the nurse cells in this region, while in the egg tubes.

Figure 32 is from a longitudinal section through the animal pole of an egg, showing a spindle with chromosomes in anaphase, within a dense granular mass, whose long protoplasmic processes reach out into the more reticular portion of the cytoplasm. This latter region is free from yolk, and is cone-shaped in form, the apex pointing inward, and extending as a sort of narrow vacuolated core, into the center of the egg. The remainder of the egg is filled with large yolk spheres. At the periphery of the egg appears a thin layer of dark granular protoplasm.

In earlier eggs, before or at the time of laying, a clear pale vitelline membrane may be seen beyond this. The polar bodies are formed in the dense granular layer, very near the middle of the anterior end, just within the cone-shaped area.

The earliest nuclear stage obtained after the growth of the egg, is the late prophase, found in eggs which had not been laid (fig. 3). The chromosomes, 13 in number, lie enclosed within the nuclear membrane, near the surface of the egg, in the same position as the first maturation spindle. The chromosomes are smooth, elliptical or dumb-bell shaped bodies, almost equal in size. Later the nuclear wall breaks down, the spindle fibers appear and the chromosomes become arranged upon them preparatory to division (figs. 4-5). When first placed upon the spindle, the chromosomes do not all show a dyad form, but later a median constriction appears in each one. The spindle lies obliquely to the surface of the egg. The spindle fibers can rarely be traced to a point of convergence, and no centrosomes nor asters appear. Various cytoplasmic bodies lie near or attached to the spindle (fig. 4), but they are not constant in size or number, and often cannot be detected. They are present only during the metaphase.

In figures 6 and 7 are shown two first division groups. There is but slight difference in the size of the chromosomes, and each one appears to be separated into two equal parts. On account of their small size they were at first interpreted as chromosomes of the second division, but a further study showed that this was not the case. There was no trace of a first polar body, nor of sperm within the egg. Moreover, the eggs had not been laid, but were taken from a moth which had just begun laying. Figures 4 and 5 and numerous other undoubted first metaphase stages, with larger chromosomes, were obtained from the same lot of eggs. Restaining and extraction had practically no effect in altering the size difference which remains unexplained. In twelve cases I found chromosome groups similar in size to those shown in figures 6 and 7. These figures seem to indicate that the chromosomes divide equally in the first division but a

definite statement is unwarranted, for the chromosomes are so small that a slight size difference might easily escape detection; moreover the variability in size in different groups, as seen in figures 5 and 6 would render any deductions in this respect extremely hazardous.

As the chromosomes approach the ends of the spindle, the fibers thicken enormously in the middle, forming a deeply staining cell plate, which in side view, gives the appearance of a band encircling the spindle (fig. 8). Henking ('92) described similar bodies in *Pieris*, which he considered as waste achromatic substance. With long extraction the cell plate appears very faint, while the chromosomes remain dark. Figure 9 shows another anaphase, in which 13 chromosomes may be counted at each pole. No lagging chromosomes were observed. In a late anaphase (fig. 10) another size peculiarity is observed, each chromosome being after division approximately as large as those of the metaphase stage. The irregularity in the form of the chromosomes in late anaphase was characteristic of this stage, and was equally apparent with either dark or light staining, although in the latter case the chromosomes appeared slightly smaller. Figure 11 is an oblique polar view of a similar stage; on account of the plane of the section the spindle cannot be seen. In figures 12 to 14 are shown polar views of spindles, the groups lettered *a* in each case being those entering the first polar body. Four chromosomes are in the center of each group, surrounded by a ring of nine. The polar body groups sometimes appear slightly smoother in outline than the egg groups (fig. 12), smaller, and bipartite in preparation for a second division. In attempting to compare chromosomes in the polar body with those in a similar position in the egg group, it is impossible to obtain any evidence either for or against an equal division of chromosomes. The variability is extreme, within both egg and polar groups, and in many cases it is very difficult to be sure of the actual chromosome outlines.

During the formation of the first polar body the spindle fibers elongate considerably, and the granular cytoplasm forms a

conspicuous projection on the surface of the egg (fig. 33). There is apparently no first telophase, for no loss of contour or massing of the chromosomes was observed between the late anaphase and the second metaphase.

c. The second maturation division

In the second division two spindles appear, as shown in figure 34. Upon one are arranged the chromosomes of the first polar body; on the other, those of the second polar division. The old spindle fibers have disappeared, and the cell plate has assumed the form of irregular deep-staining masses. At a corresponding stage in the oogenesis of *Bombyx mori*, Henking ('92) figured a cell plate in a similar position, but it differed from this in being a single disc-shaped body or 'thelyid', which stained very faintly. The later constriction of the first polar body in *P. cynthia*, as in *Bombyx*, does not involve the cell plate but passes between it and the outer group of chromosomes.

In figure 15 A, B, drawn from adjacent sections, are shown 13 approximately equal chromosomes, arranged upon the two spindles, preparatory to a second division. It will be observed that the groups in the egg B and the first polar body A are at this time similar in the form and size of the chromosomes. The remnants of the cell plate are composed of deep-staining bodies, so large and definite as to give the appearance of chromosomes, but they are very irregular in form, and vary in size from large masses to very small granules. Figure 16 is another view of a similar stage. In figure 17, a polar view from three succeeding sections, the cell plate is composed of 15 large bodies, and numerous granules. One chromosome is missing from the egg group, B.

For various reasons second anaphase stages were extremely difficult to find. A few cases, however, were obtained, which seem fairly clear. In figure 19 is shown a spindle in which 13 chromosomes are seen at one pole, 11 at the other. This latter group, which is incomplete, enters the second polar body. The 13 chromosomes of the egg nucleus are very small rounded bodies nearly equal in size. There is nothing to indicate a pe-

culiarity in behavior of any of the chromosomes. In figure 20 polar views of two groups in anaphase are shown; here 13 approximately similar chromosomes appear in each. In figure 21, a polar view of an egg group, 13 chromosomes may be counted. Figures 23 and 24 are two oblique sections through spindles in anaphase. The groups in each case have been slightly displaced. There are 13 chromosomes in each group. These examples, while not numerous, are sufficient to show that the second polar body receives a group of chromosomes similar in number to those remaining in the egg. The small size of the chromosomes, and the lack of early anaphase stages, make it impossible, as in the case of the first division, to draw any conclusions as to the equal or unequal division of the chromosomes.

The first polar body was frequently observed in anaphase, during the stage figured above. After the second anaphase the egg chromosomes show a tendency to fusion (fig. 25) and it is impossible to distinguish separate chromosomes at either pole.

d. Fertilization

In sections through eggs in the first metaphase stage, several spermatozoa may be seen within the vitelline membrane, but only occasionally within the egg. In late anaphase, sections show that the spermatozoon has penetrated into the egg and is enveloped in a dark granular island of cytoplasm. Numerous eggs were found containing two or three spermatozoa. Thus in *P. cynthia*, as in many other insects, polyspermy appears to be normal. Shortly after entering the egg, the sperm appears as a long tapering rod. Later, it has the form of an oval, deep-staining vesicle surrounded by a clear area, and in contact with the female pronucleus, which lies nearer the surface of the egg. Subsequently the male pronucleus becomes spherical, the clear area disappearing. The chromatin in both nuclei is in the form of irregular flocculent masses, at first darker in the male pronucleus. Later it has the same staining capacity in both, so that it is impossible to distinguish male and female except by position.

At a later period, it is possible to count the chromosomes in each nucleus. In figure 35 the pronuclei lie near the surface of the egg where the second polar body appears. The surface cytoplasm merges with the remnants of the vitelline membrane, in which the polar body lies. Figure 27 is the same section enlarged. The pronuclear walls appear broken at the region of contact, or are so thin as to be invisible. Nine chromosomes are seen in the first section of the outer nucleus, 11 in the first section of the inner; they differ slightly in size, and some are noticeably dyad in form. To the right of these are drawn portions of the nuclei from a succeeding section, showing 4 more chromosomes in the outer nucleus, 2 more in the inner, making 13 in each. No nucleoli are present. Within the polar body figured here remains of spindle fibers and a nuclear membrane are seen. Here too, 13 chromosomes appear. This is probably the second polar body, for the first becomes very vague after the second anaphase.

In figure 28, from an egg similar to the one described, nine or more chromosomes may be counted in the second polar body. The first polar body has apparently divided, the chromosomes in each appearing as vague granular areas. It seems probable that all three degenerate shortly after the fusion of the germ nuclei. There is no evidence that they remain included within the egg.

2. CONCLUSIONS AND COMPARISONS

The evidence obtained from the foregoing study indicates that in *Philosamia cynthia* the 13 chromosomes seen in the late prophase of the egg all divide in both maturation divisions. The male and female pronuclei at the time of their union each contain 13 chromosomes, giving the somatic number 26, which is found in the nuclei of the blastoderm. It appears to be certain that all of the eggs contain the same number of chromosomes, but the evidence for either the presence or absence of an XY-pair is not conclusive, on account of the variability in the size of the chromosomes in the metaphase and anaphase plates. In the early oogenesis, to be described later, there is no indication of

the presence of a heterochromosome, either of equal or unequal parts, and from this we might suspect its absence in later stages. Although from the totality of the evidence, it appears probable that there is no difference in the chromosome groups, the matter will have to be left an open question.

Doncaster ('12) found that in *Pieris brassicae* both the male and female germ cells contain an equally paired heterochromosome which constitutes a chromatin nucleus during the growth period. He believed that in *Abraxas* a similar condition probably prevailed, and concluded that the chromosomes here "do not provide any visible basis for the sex-limited transmission of characters." More recently, however ('13) he has found some females of *Abraxas* with 56 chromosomes, some with 55, and he believes that there is a possibility of two kinds of eggs in this form.

Until the past year, the only recorded case of nuclear dimorphism in eggs (exclusive of parthenogenetic and sexual eggs) was that of the sea-urchin described by Baltzer ('09), in which the female appeared to be the heterogametic sex. Tennant, ('12) however, discovered that in other forms the male is heterogametic. Baltzer has recently ('13) announced that the results described in his former paper are erroneous, and he is convinced that the male is the heterogametic sex. This solves the apparent contradiction within the echinoderm group, the females being homozygous for sex in all cases described.

The latest case of heterogamy in the female is that recently described by Seiler ('13) for the lepidopteran *Phragmatobia fuliginosa*. In the spermatocyte divisions, 27 small chromosomes are present, and a large one, which, though lagging somewhat, divides equally in both maturation divisions. In the first metaphase plate of the egg, 27 small chromosomes and a large chromosome, slightly segmented or lobed appear. After the first division, at one pole of the spindle are seen 27 small chromosomes and a large one; at the other pole, 28 small chromosomes and a large one. It is a matter of chance whether the polar body or the egg nucleus receives the extra chromosome. Seiler interprets the extra small chromosome as a lobe of the large

autosome which has separated from it during division, since in anaphase a small chromatin mass lies near one end of the large chromosome as if detached from it. Second divisions were not observed but Seiler believes they are probably equational. He suggests the tentative interpretation that the extra small chromosome is the X chromosome. Unfortunately, only polar views of the first division are given, and these only of late anaphase, so it is impossible to determine how the extra chromosome arises. It is possible that the separation of this chromosome (described by Seiler) may be merely a temporary condition, followed by a union with the large one at the second metaphase, thus giving similar groups of chromosomes in all the oocytes. In view of the fact that in echinoderm eggs an apparently clear case of dimorphism has been found to be incorrect, it seems particularly necessary to scrutinize carefully any evidence along this line.

EARLY OOGENESIS

A study of the early oogenesis of *P. Cynthia* was undertaken in order to determine the origin of the haploid groups of chromosomes which enter the first polar metaphase. By analogy with spermatogenesis, pairing of the chromosomes in the egg should occur before the growth period. Although the material is unfavorable for the study of oogenesis as a whole, a seriation of stages was obtained, and several points of interest were observed in regard to the differentiation of primitive ovarian cells into eggs and nurse cells, and their later relation to each other.

1. OBSERVATIONS

a. Growth of the ovary: General description

The earliest ovaries obtained were from larvae fixed the latter part of August, a few days before the spinning of the cocoon. They are pear-shaped bodies, about 1 mm. in length, slightly smaller than a mature egg. Figure 29 is a lengthwise section through a larval ovary. The oval mass of connective tissue surrounds four egg strings which take a complicated course

within the capsule. The strings open into a single slightly expanded chamber at the surface of the ovary, from which the oviduct arises. The earliest eggs are found near the opposite end of the ovary. Oogonial stages to very early eggs were found in this and similar ovaries. Figure 30 is of a January ovary, showing an increase in size and the growth of the egg strings. Two strings are broken away from the oviduct, but their points of attachment may be seen. The stages in this ovary ranged from a few spiremes to well-developed eggs, each with its five nurse cells contained in a separate chamber in the string. The ovaries of early June were practically identical in size with those of January. All the cells by this time have differentiated into eggs and nurse cells. In early July, the ovaries are markedly different. Figure 31 shows portions of three egg strings from a pupa about three days before the time of emergence. The ovary now consists entirely of egg strings with a decidedly beaded appearance due to the growth of the eggs.

b. Early stages in the development of the ovary

A description is given below of the stages in the development of the eggs and nurse cells from the oogonia to the first metaphase of the egg.

Stage a (fig. 37 a): The oogonial region containing cells in various stages of final oogonial divisions and in rest before these divisions. A polar view (fig. 2) of a metaphase plate shows 26 chromosomes. In anaphase stages no lagging chromosomes were observed, nor differential divisions, such as have been described in several insects (Buchner '09, Günthert '10).

Stage b (fig. 36): Post-oogonial nuclei. Here the chromatin assumes the form of deep-staining bodies with ragged and irregular outlines. No constant number can be counted. The cells are connected by dense protoplasmic strands or tubes, which appear to originate from the spindle remains of the oogonial divisions. Günthert ('10) figured similar connections between eggs and nurse cells in the oogonia of *Dytiscus*. In figures 41 and 42 are shown two tubes with their branches appearing to terminate in rounded knobs, which are merely the upturned

ends of the branches. The largest number of branches observed was 6 and this is probably the correct number as sections of later ovaries show that each egg cell is connected with five nurse cells.

Stage c (figs. 38 and 39). The chromosomes are transformed into smaller irregular fragments which later assume the form of pale delicate threads. There is no trace of the uncoiling of convoluted threads from the chromatin masses to form the leptotene stage, as described by Davis ('08) and Wilson ('12) in the spermatogenesis of insects.

Stage d (fig. 40): The presynaptic leptotene. The threads now appear more definite and convoluted. A few irregular clumps of chromatin may be seen, but fewer detached fragments than before. Several free ends of the spireme are visible, but it is impossible to approximate the number of threads.

Stage e (figs. 37 b and 43): The synaptic stage or synizesis. A study of this period gives most unsatisfactory results. The contraction figure seems to follow immediately upon the leptotene stage. The spireme forms a deep-staining mass closely and intricately coiled. In some animals the synaptic knot shows two kinds of threads, thick and thin, indicating a possible parasynapsis. In *P. cynthia* all parts show the same diameter throughout.

Stage f (figs. 37 c and 44): Post-synaptic spireme. The threads now begin to spread out through the nuclear cavity. A few free ends are visible. In other cases the spireme might be interpreted as continuous. It stains deeply as before, and is of the same thickness throughout. Several writers have stated that the nuclei are not enclosed at this time, the cells forming a syncytium. In many sections of this material, cell boundaries were not to be seen, but in other cases, particularly when Flemming's fluid was used, they could be traced without any difficulty. The tubes connecting the cells stain very lightly at this stage, being only occasionally visible.

From this point on, a gradual differentiation occurs between eggs and nurse cells, so that it is convenient to treat the two separately. The further nuclear changes in the nurse cells will be considered first.

c. Development of nurse cells

A condition shown in figure 45 succeeds that of the preceding figure. The spireme is spread out through the nuclear cavity, and appears to consist of about 13 segments, most of them looped, but without any indication of polarization. A small plasmosome is present. Somewhat later (fig. 46), 13 definite segments may be counted, and the loops show a tendency to straighten out into long rods. The threads are very definite, with fairly smooth outline, and appear very slightly thicker than when first opening out. The haploid number was counted in at least twenty nuclei of this period. The plasmosome is slightly larger than before, and has no chromatin associated with it, nor is there any orientation of the segments with respect to it. Toward the latter part of this stage, the nucleus increases in size, and the chromatin segments gradually become thicker and more deeply-staining, giving rise to the condition shown in figure 47. Some of the segments are in the form of curved rods, others are sharply bent. The plasmosome is larger at this time, and frequently vacuolated.

The condition of the thick threads in figure 47 is similar in general appearance to the pachytene stage of other animals, but it is probably not equivalent in its origin, since the threads are formed, not by doubling, but by a gradual widening of the thinner threads. This stage represents the pachytene period only in the sense that it is subsequent to synapsis, and gives rise to the diplotene stage.

Stage g (figure 48): The diplotene stage. A longitudinal split now appears for the first time in all the chromosomes, and shows very clearly in cells which lie directly in contact with those of the preceding period which show no split. They are differentiated from them also in length of the chromosomes, for the split threads are considerably shorter. Doncaster ('12) describes in *Abraxas* the double thread as arising probably by a bending over of the chromosome, with a separation later at the bend, but this is certainly not the case in *P. cynthia*. There is no clue whatever to the relation of these double threads to

the chromosomes of the oogonia, since the split appears de novo, and also since there is no direct evidence that the chromatin threads have conjugated in synapsis. Unfortunately, therefore, *P. cynthia* cannot be added to the list of forms in which either parasynapsis or telosynapsis has been observed. The impression gained from a study of the material is that reduction is accomplished by a simple segmentation of a continuous spireme into the haploid number of threads.

Following upon figure 48, a progressive shortening of the segments occurs, the chromosomes often appearing extremely ragged. In a few cases, the halves show a divergence at one point as if beginning to separate (fig. 49). Very rarely the threads open in the middle while remaining united at the ends, thus forming a ring, but these forms are probably accidental and due to the fact that the chromosomes are soon to disintegrate. The later condition of this stage is shown in figure 50. The threads have by this time assumed a rod-like appearance. The lengthwise split is still apparent, but the chromosomes are extremely ragged and irregular in outline. Thirteen rods are present. At about this stage in *Abraxas*, Doncaster ('12) found the egg cells differentiated. In *P. cynthia*, as will be shown, the period is somewhat earlier.

Stage h (figs. 37 d and 51). In figure 51 almost all of the rods have shortened to bipartite chromosomes, the halves being rather widely separated from each other. Frequently a constriction in each half gives a tetrad form to the chromosome. In this figure, two are still rod-like, as in the preceding stage. The large plasmosome is frequently vacuolated. In figure 52, a later condition, the chromosomes are more irregular and stain less deeply. A few of the tetrads are broken into four separate flocculent pieces; others into irregular fragments. Some chromosomes remain bipartite as before. Within the cytoplasm a dark rounded mass indicates the end of the strand or tube which connects this cell with others.

With further growth of the cell, the nucleus increases in size, and the chromatin fragments multiply considerably. Giardina ('01), Debaisieux ('09) and Günthert ('10) have figured in the

Dytiscidae a markedly regular division of tetrads, each part giving rise to a whole tetrad, this process being repeated several times. In *P. cynthia* there is no evidence of any order in the fragmentation, for there is the greatest irregularity in the size and shape of the pieces.

The period of fragmentation marks the first broad phase in the history of the nurse cells. It is interesting to note that the cells have passed through a cycle of changes as if for maturation divisions, since they show the reduced number of chromosomes. These are destined, however, only for disintegration.

Stages j to l. At the beginning of this phase, the eggs and nurse cells are practically similar in size (fig. 37 e). The egg cell increases steadily in size during the growth period. The nurse cells, although increasing for a time, do not keep pace with the growth of the egg, and become relatively smaller as development proceeds.

Stage j (figs. 37 e and 53): At the close of fragmentation, numerous small dark granules lie within the nucleus near the periphery, together with a variable number of larger round bodies, which, although stained very deeply in some sections, are very pale in others, and appear to be of the nature of plasmosomes. The cell contents appear slightly granular, or reticular, with a very darkly granular, flask-shaped area extending from the nuclei toward one end of the cell, appearing to perforate the cell wall in the form of a curved tube which enters the egg cell. Marshall ('07), Günthert ('10) and others describe similar areas, but the tubular portion is not apparent. A later stage is shown in figure 54. The nuclear wall is less easily seen on the side nearest the tubes, for the granules are thickest at this point, and lie close to the dark granular cytoplasm. In addition to this mass of granules, the nucleus contains much smaller masses scattered near the periphery, and several small plasmosomes. A thin dark band of cytoplasm often encircles the nucleus, merging with the flask-shaped portion. Beyond this the cytoplasm appears reticular. Figure 55 A shows another cell in which the nuclear cavity is indented in two regions, giving a somewhat dumb-bell shape. During these changes in the shape of the

nucleus, the plasmosomes are extremely variable. Figure 55 B is a plasmosome from a similar nucleus, very irregular in form and encrusted with chromatin granules. In some cases the granules adhere in such numbers as practically to obscure the plasmosome.

Figure 56 is typical of older nurse cells. The nuclear wall appears to be thrown into a number of folds, beset with chromatin granules, which frequently obscure the wall. The nuclear cavity contains as before, clumps of granules and plasmosomes. The cytoplasm immediately surrounding the nucleus has become much broader, forming a conspicuous dark ring which merges into the flask-shaped region. The reticular portion of the cytoplasm is smaller in extent. Frequently at this stage or later, there appear very pale delicate cytoplasmic lines in the flask-shaped region, converging down into the tubular portion, probably indicating a transfer of material into the egg cell. In figure 56 two nurse tubes are shown, the egg cell into which they open not being indicated. The tubes appear longer than in the early stages, and are irregularly constricted in places, often apparently forming a series of rings lying upon each other. There appears to be a thin homogeneous membrane forming a distinct wall to the tube; this is not a continuation of the cell wall, but is formed at the edge of the flask-shaped cytoplasm, and passes through the cell wall (fig. 54). In *Eacles imperialis* and *Telea polyphemus* a similar condition was observed, although the tubes here are not so prominent.

In figure 57 a later stage of the nurse cells is shown, drawn to the same scale as figure 56. Here the nurse cells are considerably larger than before, yet smaller than the egg. The flask-shaped region, circular granular region and nuclear cavity appear as previously indicated. The plasmosomes are covered with granules, and single strands of more prominent granules partly line and extend down into the circular region. Only two nurse cells are figured here. The total number for each egg is five, which can be readily determined by following through a series of transverse sections. Gross ('03) also found five in other Lepidoptera. In figure 57 and other similar sections, the follicle cells are arranged in a layer around the groups of eggs and

nurse cells, first definitely formed at the periphery of the egg string, then growing in at the base of the egg cell. Later they grow in between the egg and its nurse cells, separating them except in the region of the tubes.

Stage l. At the end of the growth period, when the nurse cells have become very small, no definite tubes are seen, the cytoplasm opening broadly into the egg. Later the follicle cells form a continuous layer over the egg, and the nurse cells may be seen as small degenerated masses of cytoplasm which eventually disappear.

d. Development of egg cells

The further history of the egg cells, beginning with their differentiation from the nurse cells in Stage f is given below.

Stage f: Post-synaptic spireme. Among the nurse cells of this stage (figs. 44-46) a few cells may be observed in every section, in which the spireme appears to be thicker than in the surrounding cells, more continuous and more closely convoluted, as in figure 58. The nucleus is somewhat larger, and is very frequently distinguished by a pale yellowish tinge. Characteristic of this stage is the large plasmosome, frequently surrounded by a darker rim. The cytoplasm is also slightly greater in amount. A large number of sections were examined, and these differences appeared fairly constant. Careful study of cells in the earlier contracted spireme failed to reveal any criterion by which the egg cells might be identified at this time.

After the nurse cells are well differentiated, the spireme of the egg cell appears much less convoluted (fig. 59 A), spreading out through the nuclear cavity, which has increased considerably in size. In figure 59 B is shown a portion of the spireme which was not included in the first section. It is not possible to determine accurately if the spireme is continuous, but it is my belief that this is the case. During this period, one or two large plasmosomes may appear, and frequently two smaller bodies, probably of the same nature. The entire nucleus has the yellow tinge noted in the earliest stage of its differentiation.

The cells next to be described are taken from sections of ovaries fixed in January, later than the preceding sections. Figure 57, already referred to, shows a portion of an egg string just beyond its point of emergence from the ovary proper. Figure 60 is a nucleus from a similar egg. The spireme is typical for the eggs at this period; it is still convoluted as before, with no trace of a longitudinal split. The plasmosome varies considerably in form, consisting usually of a dark spherical portion, and a light portion, sometimes lobed and vacuolated. In this figure the plasmosome gives the appearance of breaking through the nuclear membrane, and in another egg near by a similar body was observed lying in the reticular cytoplasm at a little distance from the nucleus. A few other cases were observed on the same slide. The material appeared to be unusually well fixed, but as other ovaries failed to show a similar condition, this is probably not a normal occurrence.

It is convenient at this point to note more definitely the relation between the eggs and nurse cells during this period of growth. In figure 57 two nurse cells are seen connected with the egg, the flask-shaped region with its faint converging lines of protoplasm is confluent through the nurse tubes with a dark granular layer which surrounds the egg nucleus, broadening out into a conspicuous mass on the farther side of the nucleus. Here it sends long processes radiating out into the reticular portion of the cytoplasm. In most sections this finely granular region has a yellowish tinge, like the nucleus, markedly different from the reticular region of the cells. The mass frequently contains small vacuoles and deep-staining granules and is similar in appearance to the so-called yolk nuclei in various eggs; it seems probable that in *P. cynthia* the mass is of the same nature.

Pauleke ('00), Gross ('03), and others, have described whole nurse cells entering the egg during the growth period. This would be impossible in the moth, on account of the small diameter of the nurse tubes.

Stage g: Disappearance of the spireme in the later growth period. The next stages figured are sections from the ovary shown in figure 31, from a moth fixed a few days before the time

of emergence. In a few of the youngest eggs in this material, the spireme is still vaguely discernible (fig. 61) in the form of a pale network of irregular threads joined together, not the coiled spireme of earlier eggs. Several dark bodies of irregular size and shape are characteristic of this period. In figure 62—a slightly older nucleus in the same string—all traces of the spireme have disappeared. The nuclear cavity contains a pale body with a large vacuole, and numerous smaller rounded masses, which frequently stain very deeply. These are probably all plasmosomes. There is extreme variability in respect to their number, size and appearance, some being apparently homogeneous, others filled with vacuoles. Figure 63 is a nucleus of about the same age as figure 62.

As the eggs increase in size, the nuclei appear to have at one side a darker region, frequently crescentic (fig. 64), which seems to be connected with a dark granular protoplasmic strand running down into the cytoplasmic region of the egg, now cone-shaped, as in the mature egg. The nucleus is partly surrounded by yolk spheres, lying in faintly granular cytoplasm. The crescentic region merges gradually into the lighter granular portion of the nucleus, and suggests merely a condensation of the nucleoplasm here. Over twenty-five nuclei of this stage were examined, after varying degrees of extraction. In many cases the contents of the darker region were visible, and all showed the same condition of darker granules merging into lighter ones. No plasmosomes were to be seen, nor any trace of chromatin. The nuclei lie near the periphery of the eggs in the cytoplasmic region near the nurse cells, which at this time are reduced to shrunken remnants. Later the nuclear wall seems to fade out at the side nearest the periphery, and several bipartite rod-like chromosomes may be seen within the nucleus. At a slightly later period, the chromosomes, now shorter and more dumb-bell-shaped, appear to lie in a rounded area in which no distinct nuclear boundary is discernible. In the latest prophase (fig. 3) the nuclear boundary reappears, very faint, and very much smaller than the former nuclear area.

e. Degenerating cells in the ovary

Groups of degenerating cells are to be found in almost all of the ovaries examined, occurring chiefly in the region of the tetrads. Similar cells were also noted in the spireme region, but very rarely in the egg strings. These cells have the form of clear vesicles which contain one or more deep-staining spheres of chromatin material. A number of writers have noted this condition both in oogenesis and in spermatogenesis.

No cases of amitosis were observed in any of the germ cells. The nurse cells do not divide in any manner after the last oogonial divisions, nor do the egg cells, until the maturation divisions.

f. Abnormal nuclei in the early ovary

Certain abnormal conditions were observed in nurse cells of Stage g. In two ovaries, several cells showed, instead of 13 bipartite rods, from 15 to 19 rods. These differed further in the fact that no longitudinal split was evident. As it happened that these two ovaries were the first ones examined, the problem was very puzzling, for it appeared to indicate that the chromosomes were not of the reduced number. Normal nuclei, however, were found in the same material, and sections of about 40 other ovaries failed to show any abnormal cells. Doncaster ('12) mentions a somewhat similar abnormality, in which one cell showed the diploid, instead of the haploid number of chromatic threads.

2. CONCLUSIONS AND COMPARISONS

Differential divisions in the oogonia, which have been described for the Dytiscidae, are not found in *P. cynthia*. The germ cells all appear similar in size until the post-synaptic spireme stage, agreeing in this respect with *Pieris* and *Abraxas* (Grünberg '03, Doncaster '12), the bee (Pauleke '00), and the dragon-fly (McGill '06, Marshall '07): Doncaster finds a differentiation appearing a little later than in *P. cynthia*, when the chromatin threads shorten to form bipartite chromosomes in the nurse

cells. The egg spireme is not continuous, but is composed of the haploid number of interlaced threads, which have not yet contracted. Marshall ('07) found a still later differentiation in *Platyphylax*, a neuropteran, in which the tetrad stage is common to both kinds of cells, but the egg cell is larger, and the tetrads persist longer before disintegration.

The haploid number of chromatin segments is present in the nurse cells of *P. cynthia*, as in *Pieris* (Doncaster '12), indicating a preparation for division in these cells whose function is only nutritive. A number of writers, including Grünberg ('03), Gross ('03), Marshall ('07), and Woltereck ('98), figure tetrads in the nurse cells of various animals, but do not state whether the haploid number is present. They agree, however, that differentiation of eggs and nurse cells occurs after synapsis, which would imply that nurse cells as well as eggs must have undergone pseudo-reduction.

A transfer of material takes place from the nurse cells and the egg through the connecting tubes which in *P. cynthia* have very prominent walls. A markedly similar condition was observed by Günthert ('10) in *Dytiscus*, where converging bundles of fibrils appear, beset with chromidia or chromatin granules which enter the egg. In this case there is no definite wall to the tubes. Grünberg ('03) states that in *Pieris* the egg sends a large blunt process up between the nearest nurse cells. Evidently there is considerable variation in the relation of eggs and nurse cells within the Lepidoptera, for in *P. cynthia* it is the nurse cells which send processes into the egg.

The history of the egg nucleus seems to show that the chromosomes lose their visible identity during the growth period. I am convinced of the accuracy of the results in this particular, on account of the very careful study given to this stage. More than half the nuclei from one individual were examined, and only in the very earliest eggs were traces of spiremes to be found. I examined also egg strings of other moths. In *Clisiocampa* the spireme persists relatively longer, being found in large eggs. As in *P. cynthia*, it becomes gradually fainter and more broken the older the eggs become, and finally disappears altogether.

Throughout the growth period the nucleus contains many small non-chromatic bodies, in a pale flocculent nucleoplasm, but no trace of a spireme. Sections of *Rothschildia jorulla* eggs and *Actias luna* showed an essentially similar condition.

The literature dealing with the condition of the chromosomes during the growth period contains a number of diverse results. In several groups of vertebrates and invertebrates the persistence of the chromosomes has been demonstrated by Griffin ('99), Stevens ('04), Dublin ('05), Marshall ('07, '10), Rückert ('02), Born ('94), Schockaert ('02), Winiwarter and Saintmont ('08), King ('08), and others. Deton ('09) found only a pale reticulum in the egg of *Thysanozoon*; nevertheless he believes, with Grégoire ('09) that, whatever the appearance, the chromosomes persist autonomously up to the maturation divisions. On the other hand, evidence that the chromosomes disappear as such during the growth period, is given by the work of Carnoy and Le Brun ('99), Häcker ('95), Woltereck ('98), Bonnevie ('06), Popoff ('07), Goldschmidt ('08), and Schleip ('09). The latter found an interesting condition in *Cypris*; in one form the chromosomes may be traced throughout the growth period, in another they disappear. With the second group *Philosamia cynthia* is to be included, as the facts observed indicate a gradual disappearance of the spireme during the growth of the egg.

3. LITERATURE ON THE EARLY DEVELOPMENT OF EGGS AND NURSE CELLS

In this list are included only a few of the papers dealing with the various early stages in the growth of the eggs and nurse cells in insects.

Lepidoptera. Doncaster ('12) describes the early oogenesis in *Pieris brassicae* and *Abraxas grossulariata*. In *Pieris*, after the oogonial divisions, when 30 chromosomes are seen in the equatorial plate, the nucleus enlarges and forms a reticulum, followed suddenly by the synizesis stage, in which a chromatin nucleolus appears. In the ensuing stage, a broken spireme of 14 separate threads is seen, the fifteenth element being repre-

sented by the double chromatin nucleolus, which is interpreted as an equally paired heterochromosome. When the threads shorten to chromosomes, this is indistinguishable from the others. In *Abraxas* these stages are similar. A distinction is noted here between eggs and nurse cells. In the former "the bivalent threads persist to the latest stage observed—possibly till the prophase of the polar divisions;" in the latter, the bivalent threads shorten into loops to form chromosomes. In *Bombyx* and *Pieris*, as described by Grünberg ('03), the germ cells are at first all alike, with nucleolus and granules in the nucleus. The next zone in the ovary shows spiremes, in which stage synapsis occurs. Cell boundaries are not figured here. This is followed by a differentiation zone, in which the egg nucleus is distinguished by a nucleolus and threads, the nurse cells by tetrads. Details of their origin are not given. Fragmentation of the tetrads is described, and the arrangement of nurse cells near the egg, followed by a transfer of granular material to the egg cell.

Neuroptera. The observations of Marshall ('07) on the ovary of *Platyphylax* are meagre as regards chromatin changes. From synapsis, beaded threads appear, showing a lengthwise split. These threads give rise to tetrads, which fragment. In cells destined to form eggs, the tetrads disappear later and the nuclei are slightly larger. The further history of the eggs and nurse cells is not given.

Hymenoptera. In the early ovary of the bee, Pauleke ('00) observed that the eggs and nurse cells are at first similar. Beyond the synapsis stage, the nurse cells differentiate, the chromatin fragmenting and the nucleus increasing in size. Later the cytoplasm of the egg may be seen projecting into the region of the nurse cells, which are very numerous. He believed that nurse cells entire might be taken into the egg, and that amitosis occurred.

Hemiptera. In a study of the early ovary of *Protenor*, Foot and Strobell ('11) distinguish three zones in the ovary: Zone A consists of nuclei with numerous granules; zone B of larger nuclei with granules and a nucleolus, arising by growth from A;

zone C contains very small nuclei similar to A, arising chiefly from the cells of zone B by amitosis, and giving rise to the ova. They believe amitosis plays an important rôle. No cell boundaries appear in these zones. In young ova, leptotene threads are seen, followed by a stage of broken spireme threads. These gradually disappear, and reappear later to form chromosomes. The figures given are chiefly photographs and do not adequately illustrate the points mentioned in their paper. The importance of amitosis has been questioned by Gross ('01), who concluded from his studies on Hemiptera that nuclei which divide amitotically never divide again by mitosis.

Payne ('12) also discusses the origin of the ova in *Gelastocoris*, another hemipteran. He finds the same three kinds of cells, although their arrangement in zones is very indefinite. He is convinced that the eggs are not derived from the small nuclei of zone C, but from certain larger cells of zone B, which he finds in synapsis, and that there is no break in the continuity of the cells from oogonia to ova. This appears to be a more reasonable interpretation of the development of the eggs, and accords more closely with the conditions observed in other insects.

Coleoptera. Debaisieux ('09) has described the early oogenesis in *Dytiscus marginalis*, amplifying Giardina's work on the same form. Debaisieux discovered a synaptic and a diplotene stage between the zone of differentiation of eggs and nurse cells and the growth zone. In the latter, the chromatin of the nurse cells gives rise to tetrads which fragment, the chromatin of the egg cell remaining in the diplotene stage as before. His main conclusions are, (1) that the 'chromatic mass', which Giardina believed to be derived from certain chromosomes is not true chromatin, but a condensation of the reticulum left in the nucleus after the chromosomes of the last oogonial division are formed; (2) that the chromosomes persist autonomously up to the maturation divisions. In Günthert's paper ('10) the chief point of interest is the description of differential mitosis in the oogonia. When a cell divides, a 'chromatic mass' and the spherical remains of the spindle pass into one cell undivided. This becomes the egg cell, which again divides differentially

as before. At the end of the fourth differential mitosis, there are 15 nurse cells and one egg cell, which enters the resting stage. The spindle remains of the nurse cells join that of the egg, forming a protoplasmic bridge between them. Günthert believes that differential divisions occur in many animals; and that the 'accessory body' described by Buchner ('09) in *Gryllus* is merely a 'chromatic mass' indicating a differential mitosis. The origin of nurse cells from the egg by a process of budding, as described by Will, is probably to be interpreted in the same way. In the later nurse cells of *Dytiscus* Günthert finds tetrads which subdivide regularly several times, freeing thousands of granules in the nucleus. When the nuclear wall breaks down, they migrate into the cytoplasm, where they increase by division, eventually entering the egg.

Orthoptera. Buchner ('09) figures a leptotene stage in *Gryllus* after the oogonial divisions, followed by a diplotene stage during which the 'accessory' is much vacuolated. The probable significance of this body has been referred to above. After the diplotene stage the threads shorten into rods and tetrads. No further development of the egg is given.

SUMMARY

1. In *Philosamia cynthia* the 13 bivalent chromosomes of the late prophase all divide in both maturation divisions.
2. The male and female pronuclei at the time of their union each contain 13 chromosomes, making the somatic number 26, which is found in the nuclei of the blastoderm.
3. On account of the great variations in the size of the chromosomes in the metaphase and anaphase plates, there is no conclusive evidence for either the presence or the absence of an X Y-pair of chromosomes.
4. In the oogonia no differential divisions occur. The germ cells all appear similar through the presynaptic and synizesis stages.

5. In the post-synaptic spireme stage, the nuclei of the future nurse cells show the haploid number of threads, indicating a preparation for division, although the chromosomes are destined only for disintegration. In the egg cell the spireme is probably continuous. A plasmosome is present in both cases.

6. During the growth period, the chromosomes of the nurse cells fragment into numerous granules. The nuclear wall becomes much infolded, and is lined with the granules.

7. A transfer of material takes place from the nurse cells to the egg, through connecting tubes derived from the spindle remains of the final oogonial divisions. The egg cell increases in size at the expense of the nurse cells.

8. Amitosis does not occur among the germ cells. Degeneration of cells is common in the region of differentiation.

9. The egg nucleus remains in the spireme stage throughout the greater part of the growth period. There is no indication of a segmentation into the haploid number of threads.

10. Shortly before emergence, a few of the youngest cells in the ovary show a faint disintegrating spireme. In most of the cells no trace of chromatin is present. This indicates that the chromosomes lose their visible identity during the growth period. It is impossible to demonstrate the form which the chromatin assumes during this period of its diffusion.

11. In the oldest cells of a late ovary the chromosomes reappear in the form of 13 short rods or dumb-bell-shaped bodies characteristic of the early metaphase groups of chromosomes.

March, 1914.

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All the figures (*Philosamia cynthia*) were drawn with the camera lucida. The enlargement is 2100 diameters, unless otherwise specified.

PLATE 1

EXPLANATION OF FIGURES

- 1 Metaphase from cell of embryo, showing 26 chromosomes.
- 2 Oogonial metaphase, showing 26 chromosomes.
- 3 Prophase of first oocyte division, showing 13 chromosomes.
- 4 to 7 Metaphases of first oocyte division, side view, showing 13 chromosomes.
- 8 Anaphase of first oocyte division, showing cell plate and chromosomes.
- 9 Same; 13 chromosomes at each pole.
- 10 and 11 Late first anaphase, oblique polar view. The upper groups of chromosomes enter the first polar body.
- 12 to 14 Three sister anaphase groups of the first division; *a*, chromosomes of first polar body; *b* chromosomes remaining in the egg.

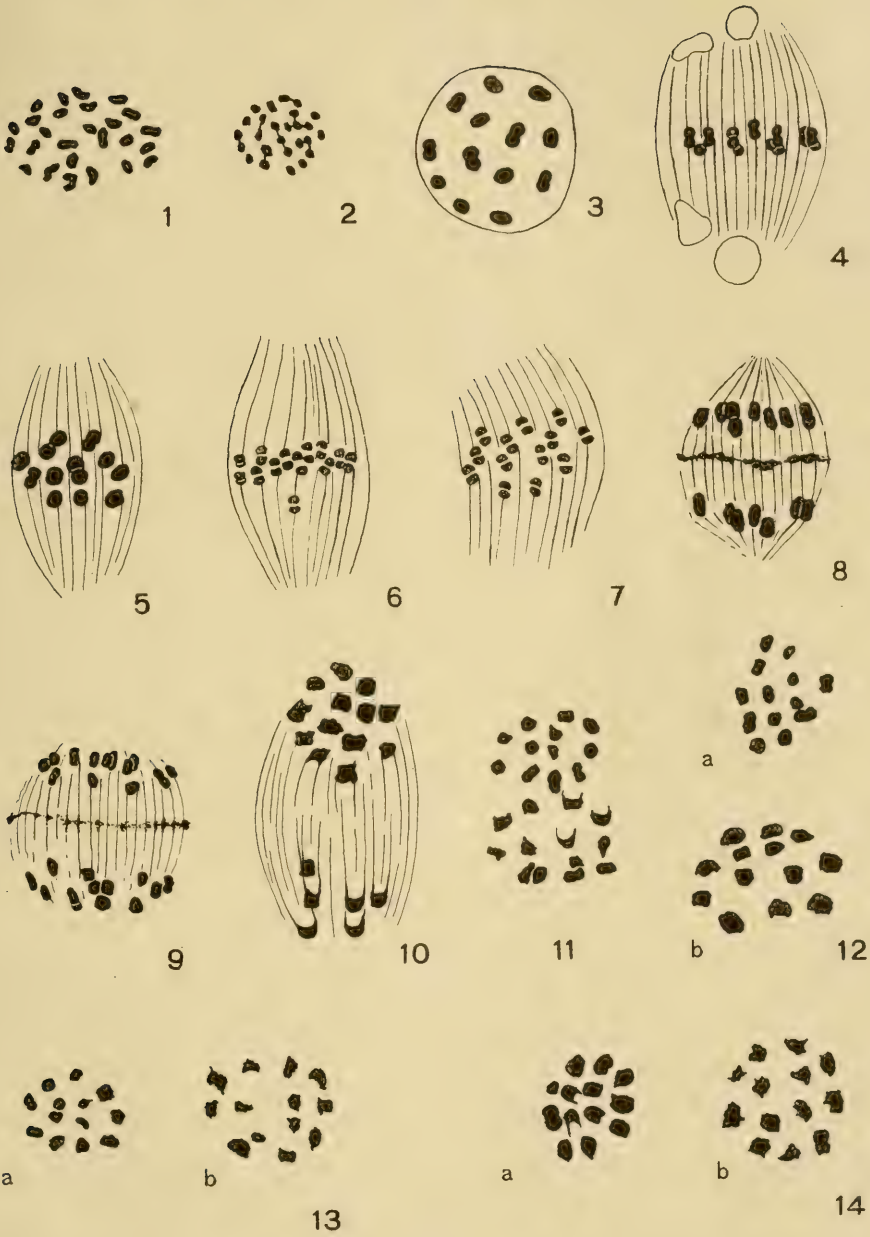


PLATE 2

EXPLANATION OF FIGURES

15 Metaphase of second oocyte division, side view, from serial sections, showing cell plate between *A*, 13 chromosomes of first polar body, and *B*, 13 chromosomes in the egg.

16 Same, without cell plate.

17 Same, with cell plate; polar view.

18, 19, 22, 26 Anaphases of second oocyte division, all incomplete except lower group in figure 19.

20 Sister anaphase groups of second division, showing *a*, 13 chromosomes of second polar body; *b*, 13 chromosomes in the egg.

21 Second anaphase group of 13 chromosomes in the egg.

23 and 24 Sister anaphase groups of second division; oblique polar view. the groups slightly displaced.

25 Telophase of second division; incomplete.

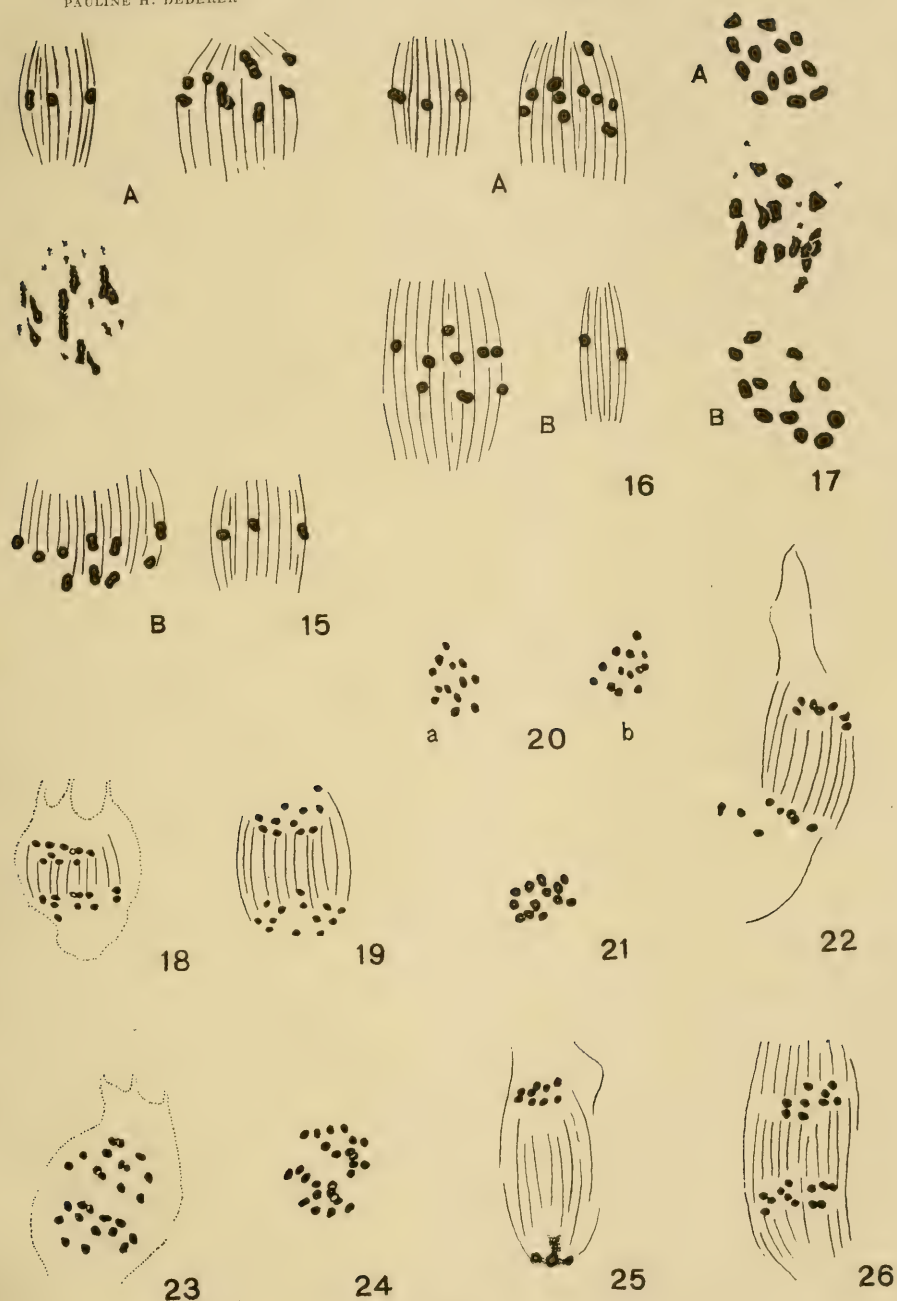
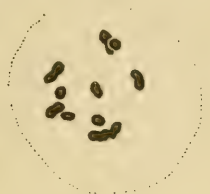


PLATE 3

EXPLANATION OF FIGURES

- 27 Copulation of the pronuclei, from serial sections, showing 13 chromosomes in each pronucleus *B*, *C*, and 13 in second polar body, *A*.
- 28 Polar bodies from egg of similar stage; the first one has divided, and shows shadowy chromosome outlines.
- 29 Ovary from a larva, longitudinal section. $\times 16$.
- 30 Ovary from a pupa, fixed in January; total, $\times 16$.
- 31 Same, fixed in July; total, $\times 16$.



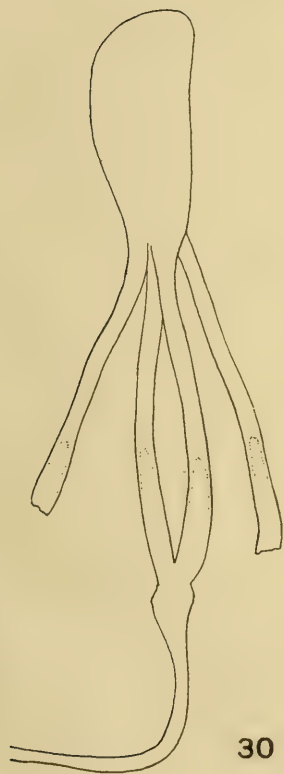
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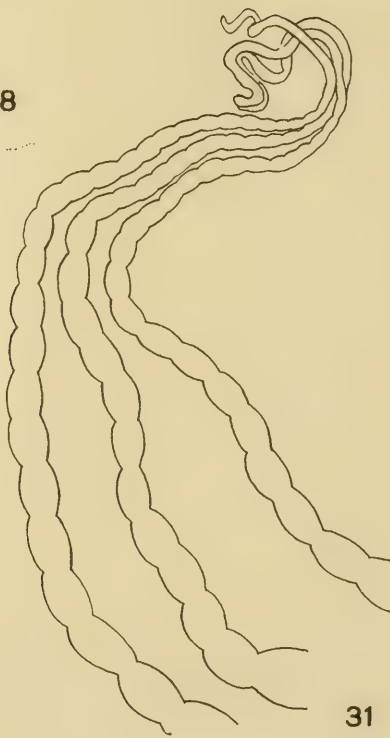
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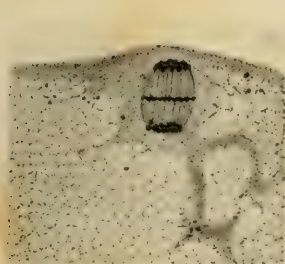


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PLATE 4

EXPLANATION OF FIGURES

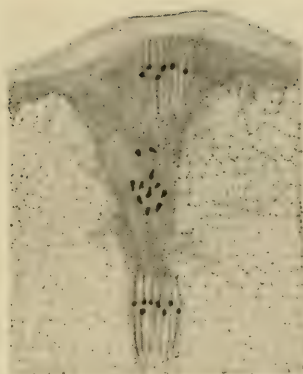
- 32 and 33 Anaphases of first oocyte division. $\times 700$.
34 Metaphase of second oocyte division. $\times 700$.
35 Copulation of pronuclei; same egg as figure 27. $\times 700$.
36 Post-oogonial nuclei with chromatin masses. The cells are connected by protoplasmic strands or tubes.
37 Longitudinal section through portion of an egg string of a larval ovary. $\times 400$. *a*, Stage a, oogonial region; *b*, Stage c, synizesis; *c*, Stage f, post-synaptic spireme; *d*, Stage h, dyad or tetrad chromosomes in nurse cells; *e*, eggs and nurse cells well differentiated.
38 and 39 Stage c; the chromatin masses are transformed into small irregular fragments which later assume a thread-like form.
40 Stage d; presynaptic leptotene.
41 and 42 Two groups of protoplasmic tubes with branches.



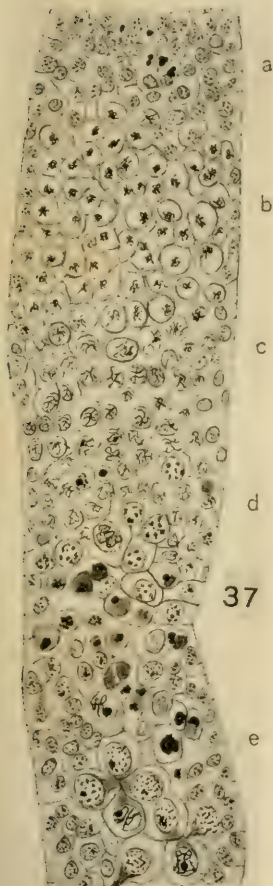
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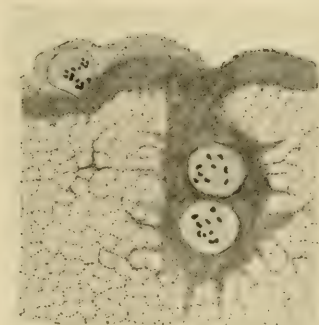
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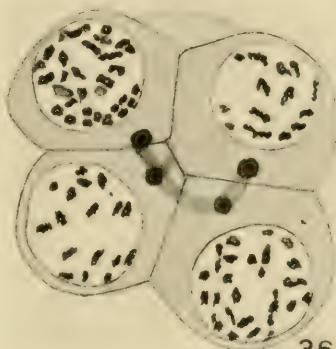
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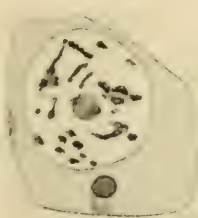
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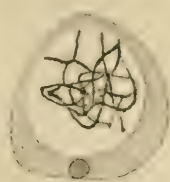
PLATE 5

EXPLANATION OF FIGURES

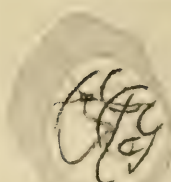
- 43 Stage e; synizesis.
44 to 47 Stage f; post-synaptic spireme of nurse cells.
48 and 49 Stage g; diplotene stage; 13 split rods.
50 to 52 Stage h; chromosomes begin to fragment.
53 and 54 Stage j; young nurse cells, showing tubes entering egg cell. $\times 700$.
55 *A*, nucleus of nurse cell with plasmosomes and chromatin granules; $\times 700$.
B, plasmosome enlarged.
56 Older nurse cells, with tubes. $\times 400$.



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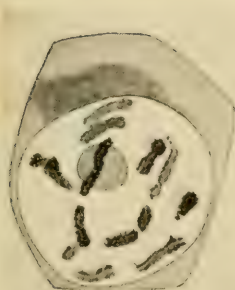
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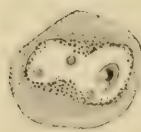
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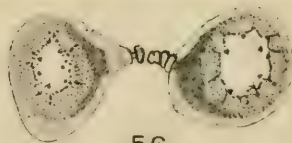


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B

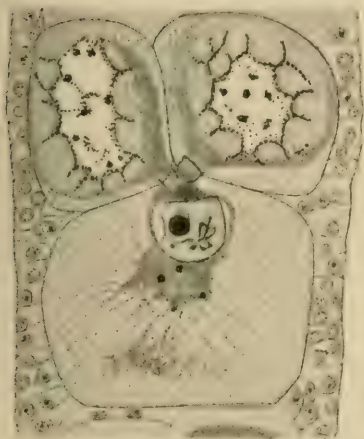


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PLATE 6

EXPLANATION OF FIGURES

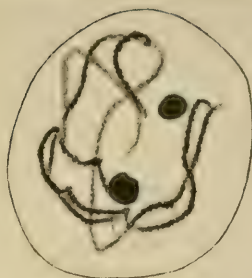
- 57 Portion of an egg string, showing an egg and two nurse cells. $\times 400$.
58 and 59 Stage f; early and later post-synaptic stages of egg cell; spireme probably continuous.
60 Nucleus from an egg cell similar to the one shown in figure 57. $\times 700$.
61 Stage g; spireme disappearing in the later-growth period; plasmosomes of varying size and form.
62 Slightly older nucleus; all traces of the spireme have disappeared.
63 Same. $\times 850$.
64 Nucleus from a nearly mature egg, showing dark crescentic region. The nuclear cavity is filled with granules. $\times 400$.



57



58



A

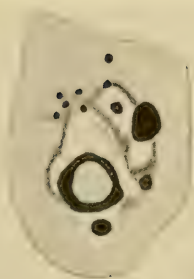
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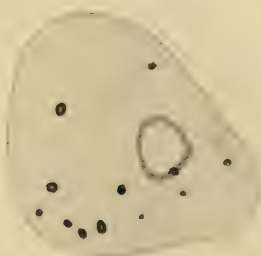
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B



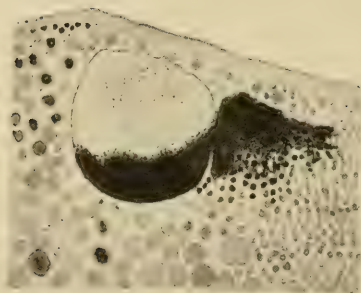
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THE STRUCTURE AND GROWTH OF THE INCISOR TEETH OF THE ALBINO RAT

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TWENTY-NINE FIGURES

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INTRODUCTION

The incisor teeth of the Rodentia have long been regarded by the zoologist as having a high value for the understanding of many of the characteristics of this order. For instance, in 1888, Cope wrote "nearly all the peculiarities of the rodent dental system and manner of mastication are the mechanical consequences of an increase in length of the incisor teeth." Tullberg ('98-'99) gives the taxonomic position of the genus *Mus*, proceeding from the more general to the more specific grouping: Rodentia, Simplicidentati, Sciurognathi, Myomorphi, Myoidei, Muriformes, Myodontes, Muridae, Murini, *Mus*. A consideration of these terms merely from an etymological view suggests the importance of the teeth and jaws in the classifi-

cation of the gnawing animals. The observations here recorded are based on the study of the cellular processes involved in the formation, eruption and growth of the incisor teeth in a single rodent form—*Mus norvegicus albinus*. An additional interest was lent to the work by the fact of the increasing use of this animal for laboratory purposes, which makes it desirable to learn the time-relations of its life-processes, as a basis of comparison in various forms of experimental studies. Although the rodent incisors have been the object of much study, few observers have carried out their observations through the complete life-history, including developmental stages and adult structure, in one form of animal and this it has been our aim to do.

HISTORICAL SURVEY

Oudet ('23) proved the phenomenon of permanent growth in the incisor teeth of rodents by cutting off the teeth at the gingival margin and observing that they were regenerated. Retzius ('37) and others noted the overgrowth of these teeth in cases of malocclusion. MacGillavry ('76) observed the rate of growth of the incisors of a rabbit by making marks on the teeth and noting the gradual advance and disappearance of these marks, as the teeth grew out and were worn away.

Questions which have called forth much study and controversy are (1) does the rodent incisor belong to the milk or to the permanent dentition; and (2) which of the three incisors of the typical mammalian dental formula does it represent. Without exception, all who have studied the first question agree that the large rodent incisor belongs to the second or permanent dentition. These same studies show that abortive milk incisors occur in a varying degree in the several families of the Rodentia; and that they are slightly, if at all, represented in the Muridae. As to the second question, Cope on palaeontological evidence decided that the large rodent incisor was I_2 . Adloff ('98) on embryological evidence confirmed this view. Freund ('92), Woodward ('94) and Stach ('10) believed it to be I_1 . Weber ('04) has given a resumé and extended bibliography of this work, up to the date of his writing.

The histology of the incisor was briefly described by Owen ('40-'45) and more completely studied by J. Tomes ('50). The latter found a considerable diversity of arrangement of the enamel prisms in the different families of the order, so that in many cases he was able correctly to refer a tooth to a particular family by a simple inspection of thin sections of its enamel. Von Brunn ('87) showed that at eruption the tip of the incisor of the albino rat is free from enamel, and Sachse ('94) confirmed this on *Mus musculus*. J. L. Williams ('96), in a comparative study of the formation of enamel, gives a number of good illustrations of the structure of the enamel and enamel-organ of the rat, prepared from microphotographs.

Ryder ('78) and Cope ('88), in harmony with their views on the "Origin of the Fittest," described the form and position of the rodent incisor as manifestations of a most efficient mechanical system; and studied the various effects on skull topography, necessitated by adaptation to this system.

The enamel organ of the albino rat was studied by von Brunn ('87) who described in some detail the differences in structure between its functional labial portion and its non-functional lingual side. He also described the early continuity of the lingual side of the enamel-organ and its later penetration by the surrounding connective tissue. Roetter ('89), studying *Mus musculus*, denied von Brunn's position in regard to the invasion of the lingual side of the enamel-organ by connective tissue, and Sachse ('94), also using *Mus musculus*, agreed with Roetter and described the continuity of the lingual portion as persisting through life.

The development of the rodent incisor has been studied especially by Roetter ('89), Sachse ('96) and Meyerheim ('98). Burckhardt ('06), in his description of the development of the persistently growing rodent incisor in O. Hertwig's *Handbuch der Entwicklungslehre* has followed chiefly Sachse's work upon *Mus musculus*. In both Weber ('04) and Hertwig ('06) are extensive bibliographies and in these may be found all references not fully given in our appended list of literature cited.

MATERIAL AND METHODS

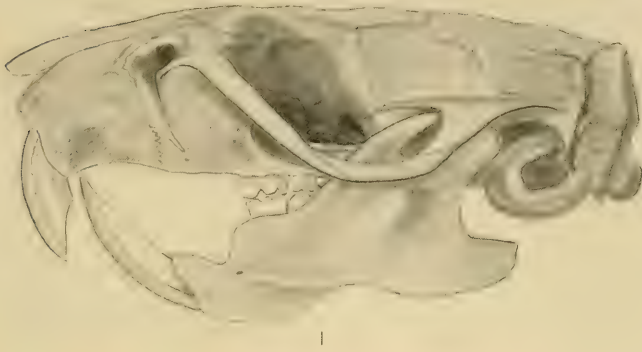
The albino rat is a variety of *Mus norvegicus*, the common gray rat (Donaldson '12). This has been shown by similarity of skull measurements (Hatai '07) and of hemoglobin crystals (Reichert and Brown '10) and also by the fact that the two interbreed freely.

The material used was obtained from the rat colony of The Wistar Institute. Serial sections in paraffin or in paraffin-celloidin were made of decalcified heads of fetuses taken at daily intervals from the 16th day onwards until birth, and of jaws of animals newly-born and at short intervals until one month, and of several older stages. Serial sections of fetuses younger than 16 days were examined in the collection of The Wistar Institute. Ground sections were made of the isolated teeth, and the petrification method of imbedding in Canada balsam was used to prepare the teeth and adjacent soft parts *in situ*. Also a series of prepared crania, some entire and some disarticulated, was made at selected ages, varying from birth to old age. The 'gold dust' method of Davison, as tested out for different ages at The Wistar Institute was used for the preparation of the former, and maceration in tap water for the latter. Schultze's clearing method was found useful in studying the early periods of calcification.

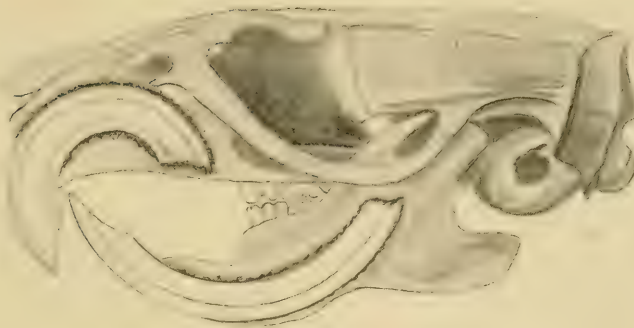
DENTITION OF ADULT ANIMAL

The dental formula of the albino rat is $I \frac{1}{1}, C \frac{0}{0}, P \frac{0}{0}, M \frac{3}{3}$.

There is only one set of teeth, and hence the dentition is monophyodont. The time of eruption of the various teeth extends over a period of $3\frac{1}{2}$ weeks. The incisors are the first to appear, viz., at 8 to 10 days after birth. The first and second molars erupt at about the 19th and 21st days respectively, and it is after this period that the young animals may be weaned and are able to maintain an independent existence, as far as food is concerned. The third molars are delayed until 2 weeks later and do not appear until about the 35th day.



1



2

Fig. 1 Cranium of a 5-month albino rat. $\times 2$.

Fig. 2 Cranium of a 5-month albino rat, with the bony alveoli dissected away to show the entire length of the incisor teeth. $\times 2$.

The incisors are permanently-growing (or rootless) teeth, while the molars have a definite limited period of development and acquire roots. A wide diastema separates the incisors from the molars as may be seen by reference to figure 1. The incisors are strongly curved and Owen ('40-'45) has described the lower incisor as being the smaller segment of a larger circle, and the upper incisor as the larger segment of a smaller circle. In the lower incisor of the albino rat this statement needs a slight modification. For while the curvature of the upper in-

cisor is in one plane only, the lower incisor is a portion of a flattened spiral, possessing a curve in three planes. The upper incisor is a segment of a true circle (at 5 months about 210°) and in cases of overgrowth it has often been known to complete the circle. In the case of the lower incisor, however, when we project it on the sagittal, frontal or coronal planes, it gives in each case a curve. It was the very evident curved projection seen on the sagittal plane to which Owen referred. Considering only this view, the lower incisor of a 5-month animal forms a segment of about four-fifths of a semicircle ($140-145^\circ$).

TABLE 1

	23 DAYS	41 DAYS	10 WEEKS	15 WEEKS	5 MONTHS	8 MONTHS	10 MONTHS
	mm.	mm.	mm.	mm.	mm.	mm.	mm.
Naso-occipital length.....	29.7	32.5	39	40	43	44	46.5
Interzygomatic.....	13.7	14	14.5	14.6	15.4	15.1	15.5
Upper diastema.....	7.4	9.5	10	11.4	12.3	12.5	13
Upper incisor—total length.....	12.8	15	18.3	20.3	23.3	23.7	26.2
Upper incisor—extra-alveolar length.....	5.1	5.5	7	8.4	8.7	9	9.3
Lower diastema.....	4.6	5	5.6	6	6.7	7	6.8
Lower incisor—total length.....	18.1	21.7	25.5	26.4	29.4	29.9	31.3
Lower incisor—extra-alveolar length.....	6.5	7	10.5	11.4	11.6	12	12.4

Measurements of the incisors and skulls of animals of different ages, were made as shown in table 1.

The teeth were measured along their convex surfaces by means of silk thread wet with water, and applied to the object to be measured. The thread was then cut with scissors at the end of the object, straightened on paper and measured to tenths of millimeters.

A consideration of table 1, shows in a definite way the peculiarities characteristic of the dentition, not only of the rat but of rodents in general. As is well known, these are the great development of the incisors, the wide diastema, and the consequent posterior position of the molar teeth as related to the rest of the skull. Cope ('88) wrote that he considered "the

increase in the length of these teeth has been due to their continued use, as believed by Ryder." The effects of this increased elongation upon surrounding parts he described under several different headings, but reference will be made here only to one, viz., upon the shape of the glenoid cavity. "A peculiarity of the masticating apparatus is the lack of a postglenoid process, and the consequent freedom of the lower jaw to slide backward and forward in mastication. Appropriately to this motion, the condyle of the mandible is extended antero-posteriorly and the glenoid cavity is a longitudinal instead of a transverse groove."

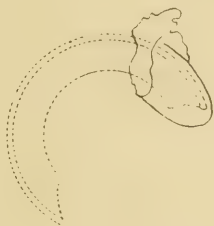


Fig. 3 Thimble-shaped portion of the maxilla bone, in which the basal end of the upper incisor is located. $\times 2$.

The lower incisors are longer and more slender than the upper and extend far back in the mandible, beneath the lower molars, to near the sigmoid notch. The upper incisors are contained within the premaxilla and maxilla, the basal end occupying a thin-walled, thimble-shaped recess of bone (fig. 3) to be seen best in the disarticulated skull, and which is attached at only one limited region to the rest of the maxilla. In both upper and lower teeth, the intra-alveolar portions are longer than the extra-alveolar. When one compares the extra-alveolar lengths of the upper and lower teeth of the mature animal, the latter are always greater, and, as may be seen by reference to table 1, the difference in lengths becomes greater with increased age and size.

In both upper and lower incisors the bone is so contoured around their imbedded portions that their course may be easily recognized. The basal end or foraminal apex of the lower

incisor forms on the outer aspect of the mandible a marked rounded projection, directed upwards and backwards beneath the coronoid process, and sometimes extending slightly posteriorly beneath the sigmoid notch. Almost directly opposite this projection on the mesial aspect of the mandible is the inferior dental foramen. This projection marks the position of the growing end of the formative organs of the incisor in the adult. In the new-born animal it is not present, nor at the end of the first month. By the age of $2\frac{1}{2}$ months it may be recognized, and thereafter it increases in prominence and constitutes a very evident feature of the bone. This region of the growing end of the tooth is protected by the zygomatic arch, and also by the overlying muscles.

The course of the upper incisor may also be readily followed in the prepared skull. Laterally it is covered with a thin rounded layer of bone. Mesially it forms an elevated, distinct ridge projecting markedly into the nasal fossa. In the adult the position of its basal or growing end is not so prominent as that of the lower incisor. As these incisor teeth are an indispensable part of the rodents' existence their importance demands protection from traumatism which might injure their growing pulp. Here in the upper incisors, this protection is afforded by a flange of the maxilla running parallel to the lateral wall of the cranium, as shown in figure 1, as well as being encased in a separate thimble-shaped recess of bone (fig. 3), beneath, and separated by a narrow interval from, the outer layer of the maxilla. These details are in harmony with Cope's idea ('88) of the influence of the incisors in moulding the general topography of the rodent skull.

The diastema in the upper jaw is always longer than in the lower (fig. 1). By reference to table 1 it may be seen that in the mature animal the upper is nearly twice as long as the lower, but that in the younger stages the difference is not so great. The upper hair-covered lips are infolded into the diastema, dividing the oral cavity into an anterior and posterior compartment. This arrangement probably prevents the débris and splinters of gnawing from entering the main oral cavity.

The mandibular symphysis is formed of fibrous tissue and allows independent rotation of either ramus with its contained tooth. This lateral movement of the lower incisors appears to be under the control of the will of the animal. According to the observations of Jolyet and Chaker ('75) this mobility has a definite purpose in mastication. They observed a rapid alter-

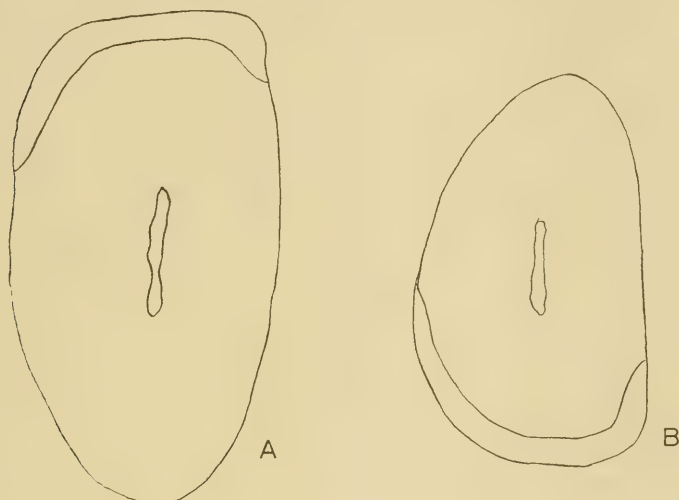


Fig. 4 Cross-sections of the (a) upper and (b) lower incisor teeth of a 5-month albino rat, taken near the alveolar margins. These show the arrangement of the enamel and the dentine, and the difference in contour of the enamel in the upper and lower teeth. The mesial surface of each tooth is towards the right side. $\times 15$.

nate separation and approximation of the tips of the lower incisors in the act of attempting to bite into a match or other slender object offered to the animal. At the same time the upper incisors were held stationary.

Mention may be made here of a point of variation among the Rodentia in the relation of the angle of the lower jaw to the sheath of bone around the lower incisor. In the Myomorphi and Sciuromorphi the angle arises from the lower surface of the incisive sheath, while in *Hystrix* the angle arises entirely on the outer side.

Ryder ('77) suggested a classification of rodents based on the shape of their incisors as seen in cross-section. In some genera the diameter of the teeth is less from side to side, than in the antero-posterior direction, while in others the reverse condition is found. The present form belongs to the former group, as is shown in figure 4. From the consideration of many rodents, Ryder deduced the general principle, that where the incisors are thicker in the antero-posterior direction, the gnawing habit is greatly developed.

MINUTE DESCRIPTION OF THE INCISORS

Enamel and dentine make up the hard tooth substance, enclosing the pulp. Owen, in his "Odontography" ('40-'45, p. 399) said that there existed a general investment of cementum over the whole tooth structure. J. Tomes ('50, p. 533) was not able to agree entirely but said that in most, if not in all, incisors of rodents cementum could be seen investing the posterior surface. In the rat, it is not apparent that there is any cementum at all. The enamel is usually colored with a pigment which is yellowish in the young but becomes orange-colored with age, and is usually more pronounced in the upper than in the lower incisors. At 13 days, there is as yet no color, but at 21 days a slight tinge of yellow is perceptible in the uppers, but none in the lowers. At 25 days the uppers are distinctly yellow, and the lowers have now acquired a slight color. At 38 days, these colors have intensified, the uppers having more pigment than the lowers; and in the mature animal the same relation continues, the uppers being orange-colored and the lowers yellow. The enamel is found principally on the labial side, and this accounts for the shape of the occlusal surface. For, the enamel being harder than the dentine, the latter is more easily worn away by the action of the opposing tooth, and the more resistant enamel remains as the cutting edge or point. The shape of the incisal end of the upper and lower teeth is different, being chisel-like (scalpriform) in the upper, and more rounded and narrower in the lower. The incisal line is also usually different in the

upper and lower teeth. In the former, it is often slightly concave from side to side, while in the latter it is convex (fig. 5).

As is shown in figures 1 and 5 the occlusal surface is an elongated concave area on the lingual aspect of the teeth, and in the living animal extends practically to the gingival margin. Due to the difference in the curve of the upper and lower teeth, the occlusal surface of the lower teeth is always longer than that of the upper, and in the mature animal it is usually found to be nearly twice as long.

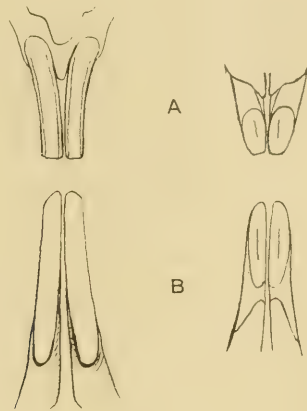


Fig. 5 Labial and lingual aspects of the extra-alveolar portions of the (a) upper and (b) lower incisors of a 5-month albino rat, showing the occlusal surfaces and incisal edges of the teeth, and the outline of the bony alveolar margins. $\times 2$.

It follows that because these teeth are constantly growing, the occlusal surfaces are constantly being worn away. As we shall see, when discussing the growth of the teeth, the elongated temporo-mandibular articulation is important, in allowing the teeth to have either the position pictured in figure 1 or to have the opposite relation, with the lower teeth outside of the upper. Thus the very important factor in the animal's economy—the proper regulation of the length of the opposing incisors—is controlled by their own inter-action.

The pulp-chamber has the characteristic shape found in all permanently growing teeth, as is well seen, for instance, in the elephant's incisor. Its cross-area is greatest at the basal end of the tooth, and gradually diminishes anteriorly. The pulp-chamber is found to extend in the tooth beyond the line of the gingivus, and very nearly to the occlusal surface. The shape

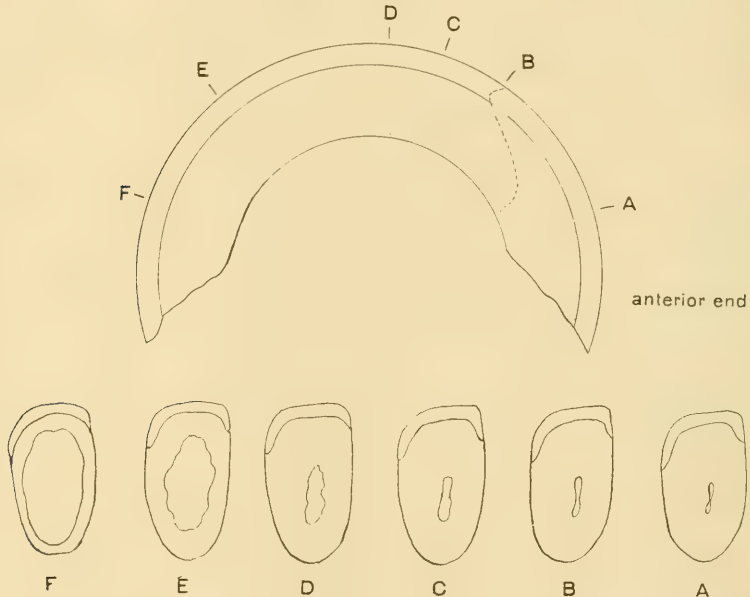


Fig. 6 Upper incisor of a 5-month albino rat ($\times 5$) and cross-sections of it at different points ($\times 8$), to show the relative cross-area of the dentine and of the pulp chamber at these regions. The dotted line indicates the position of the margin of the alveolus.

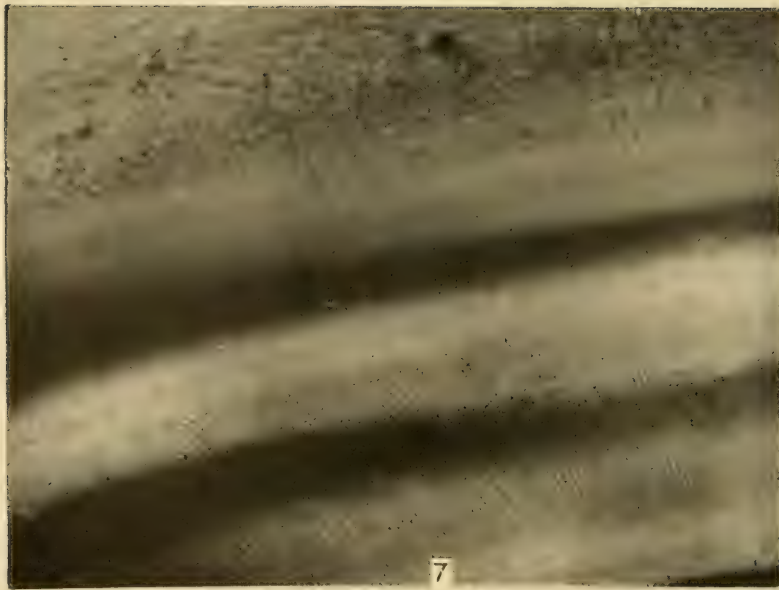
in cross-section of the pulp-chamber at different levels may be seen by reference to figure 6. The position of the filled-in pulp-chamber is usually well marked on the occlusal surfaces as a line (fig. 5). In weathered specimens of rats' teeth from recent geological formations this last-formed part which fills in the pulp-chamber at the end of the tooth, is usually found to be lacking, and is evidently not of the same hardness as the surrounding parts of the tooth.

MICROSCOPIC STRUCTURE OF ENAMEL AND DENTINE

Sections of enamel show two layers; an outer thin and an inner thicker layer, as noted by Owen ('40-'45, p. 399). The enamel rods run in different directions in the two layers as fully described by J. Tomes in 1850. In the inner layer the enamel rods appear to run in two sets, obliquely to one another, while in the outer layer the rods are all parallel. The outer layer has also been called the fibrous layer, and in its superficial part is situated the yellow or orange pigment which gives the color to the enamel.

Figures 7 and 8 show the arrangement of the enamel rods in the two layers. In the inner or plexiform layer, when examined in cross section, the alternating series of enamel rods decussate, forming an angle varying between 70° and 90° . In longitudinal sections (fig. 26) these rods are slightly S-shaped, running outwards from the enamel-dentine surface at an angle of 50° to 54° , and inclining towards the anterior end of the tooth. Figure 8 is from a ground-section in which the enamel was broken during the process of preparation, and the broken edge shows distinctly the two sets of rods running at nearly right angles to each other. Under high magnification the rods are slightly notched.

In cross-sections of the outer fibrous layer, the rods are parallel and form in the mid-line of the tooth an angle of 90° with the outer surface. As one proceeds away from the mid-line of the tooth, whether mesially or laterally, the general tendency of the long axis of the rods as they pass from the dentine junction to the periphery, is to incline in the direction away from the mid-line of the tooth. The ameloblasts usually form an obtuse angle with the rods of the outer layer and seldom coincide in direction with them (fig. 7). In longitudinal sections the rods of the outer layer are not usually so distinctly seen as in cross-sections. In favorable longitudinal sections, however, they are seen to run quite obliquely, inclining towards the apex of the tooth, and forming an angle of 20° to 25° with the plane of the enamel-dentine junction. The pigment, as will be seen



layer of
ameloblasts

outer layer
of enamel

inner layer
of enamel



Fig. 7 Portion of cross-section of lower incisor with enamel-organ, prepared by the petrification method, showing the decussation of the enamel-rods in the inner or plexiform layer and their parallel arrangement in the outer or fibrous layer. $\times 350$.

Fig. 8 Small piece of enamel, showing the rods of the inner or plexiform layer running in two directions nearly at right angles to one another. $\times 350$.

below, is confined to the outermost part of the fibrous layer. There appears to be no Nasmyth's membrane over the enamel, which means that there has been a complete transformation of the enamel matrix into enamel rods. The pigment extends about two-thirds of the total length of the upper tooth, and about one-half of the total length of the lower tooth, and hence it follows that the deposition of enamel is completed within the basal third of the upper and the basal half of the lower tooth. By examining cross-sections of the tooth at different regions (fig. 6) it would seem that the full thickness of the enamel is attained within even a smaller area at the basal end of the tooth.

The arrangement of the enamel over the labial aspect of the upper and lower teeth is shown in figure 4, drawn from cross-sections of the teeth of a 5-month animal. In both teeth the sections were made just posterior to the alveolar border. In both upper and lower teeth the enamel is thickest over the labial aspect, and is continued over the adjacent mesial and lateral surfaces. In both, the enamel is continued farther on the lateral than on the mesial surfaces, and relatively farther on the lateral surface in the lower than in the upper tooth. In the upper tooth the enamel has a flattened external surface labially, while in the lower it has a rounded contour. In the upper there is a distinct labio-mesial and a labio-lateral angle, the enamel being somewhat thicker at the former. In the lower there is a labio-mesial angle, though less prominent than in the upper, and the labio-lateral angle is practically absent.

In a 5-month animal the thickness of the enamel and its constituent layers was measured in the mid-line of the teeth, as follows:

	<i>Upper</i> μ	<i>Lower</i> μ
Total thickness.....	100-110	140-150
Outer fibrous layer.....	30-40	20-30
Pigmented portion of outer fibrous layer.....	8-10-12	6-8
Inner plexiform layer.....	70	120-125

It will be observed, however, in figure 4 that the enamel is not thickest in the mid-line of the upper tooth, but at the lateral and mesial angles. While the enamel of the upper tooth meas-

ures only 100 to 110 μ in the mid-line, it measures 160 to 180 μ at the region of these angles, and is, therefore, thicker here than the enamel of the lower tooth. The increased thickness at the angles is principally in the inner plexiform layer, the other layer being increased only slightly or not at all. The outer fibrous layer is distinctly thicker in the uppers and has a slightly wider band of pigment in it superficially. This, no doubt, is the basis of the more deeply pigmented appearance of the labial surface of the upper as compared with the lower teeth.

The dentine, unlike the enamel, grows continually thicker as one passes towards the outer end of the tooth. At the basal, growing, end it begins as an extremely thin layer. The thickness at different points is seen in figure 6. As the dentine increases in thickness, the pulp-chamber is in consequence proportionately reduced. At the distal end there is no longer any pulp-chamber and the site of its previous position has been filled in by the formation of a kind of secondary dentine. C. Tomes ('14) notes that "in some rodents the final closure of the axial tract takes place almost by a continuance of the formation of normal fine-tubed dentine, with very little secondary dentine of different structure, while in others there is a large area of dentine with vascular tracts in it." In the rat there is relatively little of this secondary dentine. It is laid down in irregular trabeculae, with the pulp tissue, including blood-vessels, at first within it. At the exposed surface, however, it forms a continuous granular mass with apparently no soft tissues in it (fig. 27). The ordinary dentine of the tooth is quite typical in structure, with numerous parallel dentinal tubules, each having many fine lateral branches. The tubules are slightly sinuous, and the lateral branches anastomose with those of neighboring tubules. Sometimes a tubule sends off at an acute angle a branch nearly equal in diameter to the continuation of the main tubule. This is usually in the dentine not covered by enamel. Where these large branches come off the diameter of the tubule is greater than elsewhere, measuring nearly 2 μ . Elsewhere the diameter varies from 1 to 1.7 μ . Slight differences may be seen between the tubules (a) in the dentine covered by enamel, and (b) in the dentine free from

enamel. The tubules of the anterior region (a) of the dentine, covered by enamel, are more regularly parallel and have finer lateral branches than elsewhere. They also seem to taper slightly as one follows them towards the enamel. In the dentine not covered by enamel (b) the tubules are more sinuous and irregular, the irregularities marking the position of origin of the larger lateral branches. In all parts at the periphery of the dentine the tubules end in a great number of very fine anastomosing arching branches. As a consequence of the smaller diameter of the little tubules here, a narrow zone at the periphery of the dentine has usually a more homogeneous appearance than has the remainder. Towards the anterior end of the tooth, in the vicinity of the pulp-chamber, are vascular channels in the form of loops within the dentine. The tubules must necessarily take a curved course around these vascular channels, and thus the position of the vessels is more easily seen.

In the dentinal tubules Mummery ('12), Fritsch ('14) and others have demonstrated not only the processes of the odontoblasts, but also fine non-medullated nerve fibers. As to why the exposed dentine on the lingual aspect of the teeth is insensitive, there are no definite observations to decide. A contributing factor may be the compression which the pulp tissues undergo at the anterior end of the pulp-chamber, leading to the physiological cutting off of the nerve supply to the dentinal tubules.

DEVELOPMENT OF THE INCISORS

The times of the early stages of development of the incisors were seen as follows:

- 14-day fetus—slight thickening of oral epithelium
- 15-day fetus—distinct thickening and growth inwards of oral epithelium
- 16-day fetus—dental ledge and beginning of flask-shaped enamel organ
- 17-day fetus—dental papilla with crescentic enamel organ capping it
- 19-day fetus—both ameloblasts and odontoblasts differentiated
- new-born animal—enamel and dentine formation begun
- 8 to 10 days—eruption of the tooth

Throughout life growth continues, and in the adult animal is on the average 2.2 mm. per week in the upper and 2.8 mm. per week in the lower incisor.

The structures to be described here, as in the development of the crowns of all teeth, are the enamel-organ with the ameloblasts, and the dental papilla (which becomes the pulp-substance) with the odontoblasts. There are two factors, however, which alter the usual history of the development of these structures, and especially of the enamel-organ. First, in permanently growing teeth of which these are examples, all these structures continue functional throughout life, so that the enamel-organ is also a persistent structure. The other factor and one correlated to some extent with the first, is that the enamel is formed on one side of the tooth only, and here only does the enamel-organ develop to its most highly differentiated functional condition.

The history of the development and growth of the tooth may be conveniently considered in two stages (1) pre-eruptive, and (2) post-eruptive. The pre-eruptive stage extends from the 14th or 15th day of fetal life until eruption of the tooth takes place between the 8th and 10th post-natal days. Until near the time of birth there is no formation of enamel and dentine, but from birth onwards these substances are laid down rapidly, so that at eruption, the teeth have their characteristic elongated narrow form. This pre-eruptive stage is characterized by the rapid elongation of the tooth-forming organs, and by the teeth attaining very similar relations to the other structures of the jaw which the imbedded portions of the erupted teeth possess. Thus, the anlage of the lower incisor appears under the oral epithelium in the anterior region of the mandible, and grows continually backwards, until its growing end reaches the region beneath the developing molars. At this time the growing end presumably reaches a region which, by reason of its increasing calcification, offers resistance to further progress. The result of the ever-continuing mitotic division and cell growth at the basal end, is the pushing of the whole tooth and its formative organs, in the opposite direction, and the consequent eruption of the tooth. During the latter half of this pre-eruptive stage, the anterior tip of the developing tooth structure is immediately beneath the oral epithelium, and remains at a fixed

point, while the posterior end is continually growing backwards and changing its relations. At eruption this condition changes, and the posterior extremity becomes practically a fixed point from which the whole tooth moves forward. That there is, however, a gradual change in the position of the posterior end of the tooth may be seen in figure 9. As the jaw grows, the entire tooth not only grows to keep the same general relative position, to surrounding structures, but it may be seen that the growing end progresses gradually posteriorly. In the full-grown animal this end occupies a distinct outpushing of the bone (fig. 1).

During the post-eruptive period, which continues throughout life, this outward growth is continued at a regular rate, and at the same rate the outer end has to be worn away. This wearing-away process would soon result in the pulp becoming exposed were not the occlusal end of the pulp-chamber also being continually filled in. As may be seen from figure 6 the dentine continues to increase in thickness until near the end of the tooth. This means that the odontoblasts continue their regular functional activity until near the end of the tooth. However, the final filling-in of the pulp-chamber to form a continuous hard occlusal surface is accomplished by the deposition of a hard matrix between the pulp elements and by the probable calcification of the latter. The result is, that as the tooth is worn away, the soft pulp never becomes exposed. Although the pulp reaches very near to the end of the tooth, a hard substance always fills in the end of the pulp-chamber, and so protects the pulp beneath.

DETAILED DESCRIPTION OF DEVELOPMENT UP TO THE TIME OF ERUPTION

The anlage of the enamel-organ of each incisor arises as an epithelial ingrowth, distinct and separate from that for the molars.

In frontal sections of the 14-day fetus, there are slight diffuse thickenings of the oral epithelium in the four positions, which represent the sites of the future tooth-formations.

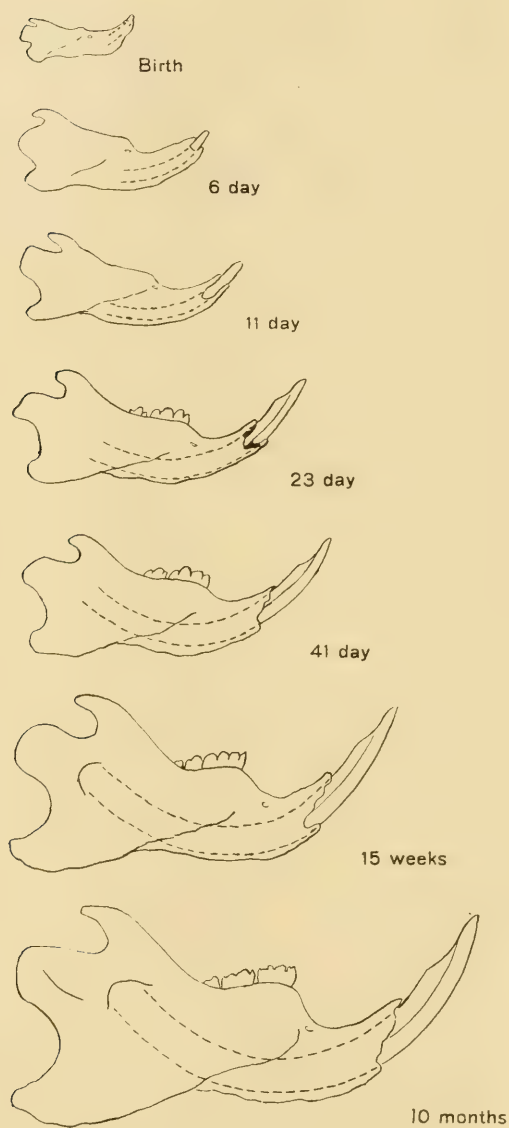


Fig. 9 Series of mandibles of the albino rat at ages varying from birth to ten months, viewed from the lateral aspect. These show the changing relation of the basal end of the incisor to the rest of the mandible during this period.

At 15 days these thickenings have become more definite, and in the lower jaws especially have begun to push into the underlying mesenchyme, and may be described as the dental ledges or dental laminae.

At 16 days the ingrowths have continued to increase as broad masses of cells, pushing deeper into the underlying mesenchyme, and in the lower jaws the enamel organs may be distinguished as expanded structures, each connected by a slightly narrower mass of cells with the oral epithelium. In the upper jaws the differentiation of the enamel-organs from the remainder of the epithelial ingrowth is not so marked.

At 17 days (fig. 10) the dental papillae are beginning, and the enamel-organs in both upper and lower jaws have a crescentic outline. In the enamel-organs there is already an indication of the differentiation into three layers. As seen in sagittal sections, the papillae develop on the posterior side of the enamel-organs, thus foreshadowing the axis of growth of the tooth-forming organs in the antero-posterior direction.

Eighteen-day fetus

Series of frontal sections of 18-day lower jaws, show that the enamel-organs are growing over the dental papillae more rapidly on the labial and lingual surfaces than elsewhere, and extend more posteriorly on these surfaces. There are thus two projections of the posterior margin of each enamel-organ as already noted by Meyerheim ('98). The labial process is broad and thin and extends more posteriorly than the lingual process, which is somewhat narrower and thicker. One may here remark, therefore, an early difference between the labial and lingual part of the enamel-organ. Other differences which will soon appear have not yet developed. Thus, the inner layer of the enamel-organ is made up of columnar elements which are still similar in all parts, both labially and lingually. In the dental papilla no columnar odontoblasts are yet seen.

The enamel-organ remains connected with the surface epithelium by a broad band of epithelial cells. In the lower jaw,

immediately laterad to the line of junction of this stalk of the enamel-organ to the surface epithelium, the lip furrow is a depression, the plane of which is continued into the underlying mesenchyme by an ingrowth of surface epithelium several layers of cells in thickness. It is by the subsequent splitting of this epithelial layer into two, that the separation of the lip will be effected.

Nineteen-day fetus

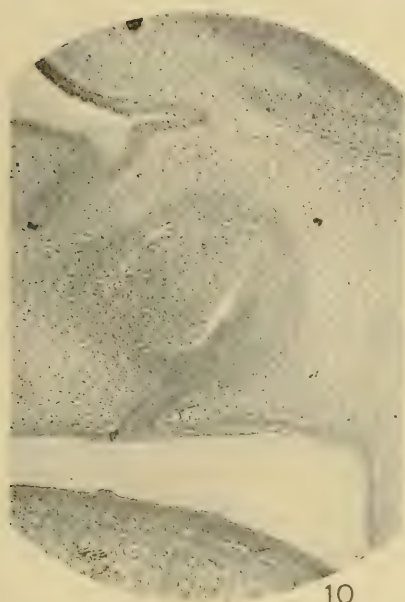
At 19 days, the enamel-organ in the upper jaw (fig. 11) is crescentic in outline in sagittal section, and in the lower jaw (fig. 12) is more elongated and conical in shape. At this age odontoblasts are first seen as columnar cells on the labial aspect of the mesodermal papilla. Three layers are recognizable in the enamel-organ, but the middle layer (enamel pulp), as has been also described by Sachse ('94) for the mouse, is extremely thin, and therefore is not present in the great quantity typically seen in the development of rooted teeth. It appears as a more lightly stained zone between the inner and outer layers, and is thickest at the basal end of the enamel-organ as shown in figures 11 and 12. It averages about 20μ in thickness and is made up of stellate cells loosely arranged. Already there is an indication of a compact arrangement of the two or three rows of cells next the inner layer of the enamel-organ, which will result in the so-called stratum intermedium seen at later ages. This middle layer is also slightly more abundant at the anterior end in the region where the enamel-organ is continuous with the stalk which joins it to the oral epithelium.

At this age the enamel-organs in the lower jaws have a greater total length than those in the upper, and especially in the lower

Fig. 10 Longitudinal section of upper jaw of 17-day fetus, showing tooth anlage of incisor, with the enamel-organ longer labially than lingually when measured from the point of junction of the stalk of the enamel-organ. The dental papilla is on the posterior aspect of the enamel-organ. $\times 70$.

Fig. 11 Longitudinal section of upper incisor anlage of 19-day fetus of albino rat, showing the crescentic outline of the enamel-organ, its greater length labially than lingually, and its thickened basal margin. $\times 70$.

Fig. 12 Longitudinal section of lower incisor anlage of 19-day fetus of albino rat, showing the conical outline of the enamel-organ and its greater length than in the upper jaw at the same age. $\times 70$.



jaws distinct differences may be made out between the oral and labial sides of the tooth-forming organs. These differences are:

(1) The enamel-organ is longer labially than on the oral side.

(2) The staining of the inner layer of the enamel-organ on the labial side is more intense, and here the cells are slightly longer than in other parts of the enamel-organ, measuring 24μ in length and assuming the typical appearance of ameloblasts. Measurements show the similar cells on the lingual side to be about 20μ in length. It is also to be noted at this age that the site of the most advanced cells which are differentiating to become ameloblasts is not at the apex of the enamel-organ, as is the case in the development of rooted teeth. For as one follows the cells of the labial side of the enamel-organ from the apex towards the base, while at the apex the cells are columnar they become longer as one goes posteriorly, and then towards the base of the enamel-organ diminish again. So that the site of most advanced differentiation here is a short distance posterior to the apex on the labial side. This is true also of the developing odontoblasts which are longest opposite the tallest ameloblasts.

(3) The outer layer of the enamel-organ on the labial side is becoming slightly wavy in outline, and this denotes the beginning of the papillae, which form such a characteristic part of the mature functional enamel-organ (fig. 26).

(4) The odontoblasts are seen only on the labial side of the dental papilla.

Mitoses are abundant in all parts of the developing tissues.

Twenty-one-day fetus

Thus the anlage of the rodent incisor begins in the usual way, and for a short time continues along the typical mammalian course. From 19 days onward, however, the differences which have already begun, become more distinct and definite. At 21 days (end of gestation) the enamel-organ has become more definitely differentiated into a labial and a lingual region. Of the three constituent layers, the inner especially is strikingly different

in these two parts. On the labial side at the anterior end, the organ has advanced to the condition where functional activity is beginning, while the oral side has remained stationary, or has actually retrogressed. Thus in the innermost layer on the labial side of the lower incisor, where the ameloblasts have begun to form enamel, these cells measure 30 to 34μ in length, while the non-functional cells on the oral side of the innermost layer are low columnar or cubical in shape and measure only 12μ in length (fig. 13). Comparison of these measurements with those at 19 days shows that the cells of the inner layer of the labial side of the enamel-organ have advanced in length from 24μ to 30; or 34μ , while the cells on the lingual side have decreased from 20 to 12μ . There is, therefore, a primary tendency for the cells of the inner layer to develop equally in all parts, but very soon the non-enamel-forming cells of the lingual side begin to retrogress, while the functional cells of the labial side continue to grow. This constitutes another point of contrast with the development of the crowns of rooted teeth. For here in the 21-day fetus, when the enamel and dentine formation has just begun, these substances are thickest, not over the apex of the tooth-forming organs, as in the usual method, but at a short distance posterior to this point, on the labial surface. Thus, not only are the odontoblasts and the ameloblasts first differentiated on the labial side, posterior to the apex, but at this region enamel and dentine formation is also evidently first begun.

Over the apex of the dental papilla there is apparently a very thin outline of dentine deposited, but within this, in the tissues of the apex of the dental papilla, there is also beginning an irregular formation of a hard matrix. Between the cells of the pulp, trabeculae of a bone-like material are appearing. As development proceeds this substance increases until the final result is, as seen in figure 20, that the primary apex of the tooth has a bone-like structure, consisting of cells imbedded in lacunae within a dense matrix. This has been called by Tomes ('04) 'osteo-dentine.'

A similar difference between the labial and oral sides is noted in the cells on the margin of the dental papilla, which are be-

coming odontoblasts. In the basal half of the papilla (fig. 14), odontoblasts occur only on the labial side opposite the tall ameloblasts, the peripheral cells of the other sides being still irregular or cuboidal in shape. Farther forwards the odontoblasts are found also on the lateral and mesial surfaces of the dental papilla, but not on the lingual. In the apical one-fourth of the dental papilla odontoblasts occur all round, measuring 20 to 24μ in length, and are engaged in the formation of dentine (fig. 13). The dentine is thickest on the labial side.

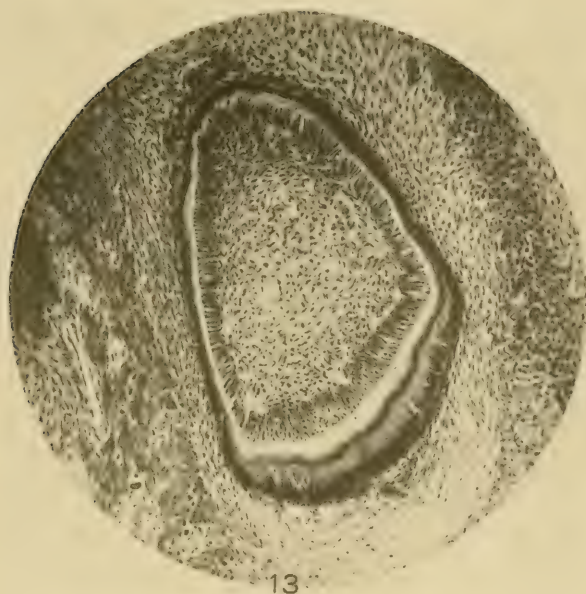
In the region where enamel and dentine formation has begun no mitoses were seen in the formative ameloblastic and odontoblastic cells, but posteriorly, where the deposition of enamel and dentine has not yet commenced, many mitoses occur in the layers of developing ameloblasts and odontoblasts, as well as elsewhere. The nearer one approaches the basal margin of the enamel-organ the more numerous are the mitoses and it is apparent that it is principally in this region that growth by addition of new cells is taking place.

One day old

At the end of the first day of post-natal life, there has been great progress in the enamel and dentine formation, and the narrow, pointed outline of the tooth has been already laid down. In the upper jaw the teeth measure about 2.3 mm. in length and in the lower jaw about 3 mm. Definite changes in its relation to the oral epithelium have occurred also at the anterior end of the tooth. The original epithelial stalk connecting the enamel-organ with the oral epithelium has increased in size and the end

Fig. 13 Cross-section of developing lower incisor of 21-day fetus of albino rat, nearer the anterior extremity of the tooth than figure 14. Shows the greater thickness of the labial side of the enamel organ, as compared with that of the other sides, and shows odontoblasts around the entire periphery of the pulp. Enamel and dentine formation has begun. $\times 110$.

Fig. 14 Cross-section of developing lower incisor of 21-day fetus of albino rat, posterior to the region shown in figure 13. No enamel or dentine yet formed at this point. Odontoblasts highest on the labial aspect, decreasing in height laterally but not yet differentiated as columnar elements on the lingual side. Enamel-organ thickest on the labial side. $\times 110$.



of the tooth has apparently advanced somewhat into it. So this thick stratified layer of epithelium forms a close-fitting investment about the tooth apex, and is continuous posteriorly with the remainder of the enamel-organ. But in this epithelial cap there are no ameloblasts and consequently there can be no enamel over the osteodentine which forms the tip of the primitive tooth. This substance forming the tip of the unerupted tooth is a form of secondary dentine with its cells located in the lacunae of the matrix. Passing backwards, one comes to the ordinary dentine containing the vascular pulp with odontoblasts situated at the periphery of the pulp-chamber in a regular manner.

As the odontoblasts were first differentiated labially, and dentine formation began there before on the other side, the dentine of the labial side is thicker than on the lingual side. Thus at a point about the middle of the entire tooth structure, the dentine measured 54μ labially and 20μ orally (fig. 15). Between the odontoblasts are numerous fine capillary loops. At this region may also be seen the characteristic structure of the enamel-organ (fig. 15). This extends all around the tooth, but is much thicker on the labial side than elsewhere. This difference in thickness is seen in all the constituent layers. In the inner layer, the tall ameloblasts of the labial surface measure 40μ , while the similarly situated cells on the other surfaces are cubical and measure only 10μ . Comparing these with the previous stage described, it is seen that the cells on the labial surface have increased and those on the other surfaces have decreased. Of the middle layer on the labial side, the stratum intermedium is a distinct line of cuboidal cells, one to two rows in thickness, lying behind the ameloblasts. The other constituent—the original enamel pulp—is small in amount and is principally within the elevations of the outer layer, which form the beginning of the epithelial papillae. The cells of the outer layer, somewhat irregular in shape with round nuclei, are in a single row. Between the developing papillae (called by Sachse Stützpapillen) are numerous capillary blood-vessels. On the other surfaces, practically nothing remains of the middle layer,

although the outer layer still persists as a layer of flattened cells. Thus lingually the enamel-organ is represented by only two rows of cells—one representing the inner, the other the outer layer of the enamel-organ.

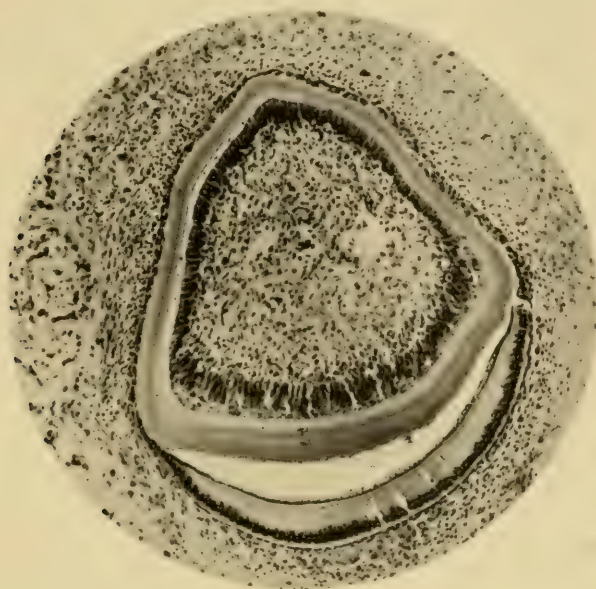


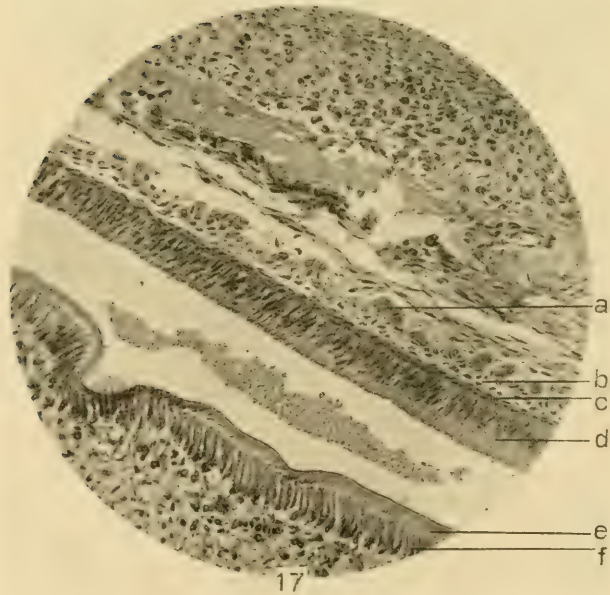
Fig. 15 Cross-section of developing lower incisor of 1-day albino rat, showing the great development of the ameloblasts on the labial side, and the thinness of the enamel-organ elsewhere. The space between the ameloblasts and the dentine is an artefact, and was formerly partly filled by the enamel, which has disappeared in the process of decalcification. In the layer of odontoblasts are seen the nuclei of the endothelial cells of the walls of capillaries. $\times 110$.

Two days old

Figure 16 shows a longitudinal section of a 2-day upper incisor. The epithelial enamel-organ is continuous over the whole tooth, but only shows its specialized functioning structure on the labial side. On the lingual side it is still intact and consists only of two rows of cuboidal or flattened epithelial cells. On the labial side, along the region where enamel has been formed (fig. 17) the



16



17

Fig. 16 Longitudinal section of upper incisor of 2-day albino rat showing the enamel-organ continuous over the labial surface and terminating posteriorly in the thickened margin. $\times 18$.

Fig. 17 Small portion of preceding figure more highly magnified, to show the structure of the enamel-organ and the odontoblasts. a, outermost layer of enamel-organ and epithelial papillae; b, enamel pulp; c, stratum intermedium; d, layer of ameloblasts; e, layer of dentine; f, layer of odontoblasts. $\times 175$.

ameloblasts measure about 40μ . These are backed by two rows of darkly staining flattened cells composing the stratum intermedium. Next to these is the looser arrangement of stellate cells, comparable to the enamel pulp of ordinary tooth development, but with much smaller spaces between the cells. This tissue is covered by the layer of cells constituting the outer layer of the enamel-organ, and the two together constitute the epithelial papillae. At the summit of each of these papillae the cells of the outer layer are grouped in a more compact manner. With higher magnification processes can be seen running from the ameloblasts into the developing enamel—the so-called enamel processes of Tomes.

At the basal formative part of the enamel-organ the three original layers show distinctly. At the thickened basal margin of the enamel-organ, around its entire circumference, is a mass of rapidly dividing cells. As seen in figure 16 this thickened margin is more noticeable on the labial side. Its peripheral zone as seen in longitudinal sections is deeply staining and its cells, more or less columnar in shape, are compacted together. The interior, of more lightly stained appearance, is composed of oval or elongated cells, irregularly parallel, but more loosely arranged than the cells of the periphery. This region constitutes the site of origin of the cells of the ever-forming enamel-organ. From this pass forward the outer and inner layers, and between them, in larger quantity than is found more anteriorly, the tissue of the middle layer. This for a short distance is all enamel pulp and shows no differentiated layer of stratum intermedium.

In this formative region on the labial side, the inner layer consists of columnar cells, the future ameloblasts, in which many mitoses are seen. While the outer layer consists of cells which are columnar near the margin, a short distance anterior to this (150μ) they change shape, first to cubical, then to flattened cuboidal. Between the two layers are cells representing the enamel pulp. At this region there are no papillae, although numerous blood-vessels are seen alongside the outer layer of the enamel-organ. About 0.5 mm. from the basal end this outer

layer of the enamel-organ becomes sinuous, and low papillae are being formed.

On the lingual side, the structure of the basal end of the enamel-organ is similar, but somewhat simpler. Thus there are three layers at and near the basal margin, but soon, proceeding anteriorly, these become reduced to two by the disappearance of the middle layer. The lingual side then consists of two rows of cuboidal or flattened cells, one constituting the outer and the other the inner layer of the enamel-organ in this situation.

The dental papilla is made up of closely packed small stellate cells, with rounded nuclei. The mesenchymal cells which lie against the basal margin of the enamel-organ are rounded or irregular in shape, but within a short distance (0.5 mm.) anterior to this margin, the peripheral cells become first cubical and then columnar in shape. Where they are beginning to form dentine they measure 30μ in length. From the odontoblasts processes enter the dentinal tubules of the dentine. The outer surfaces of the odontoblasts from which these processes arise show a distinct cuticular margin. Between the odontoblasts at short intervals capillaries form loops around the cells. These are evidently for the purpose of insuring an ample blood supply to these functionally active cells.

Four days old

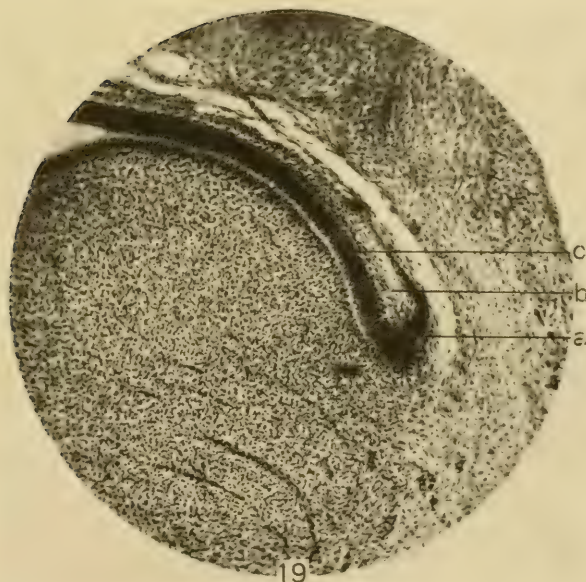
By 4 days of age there has been continued growth, and deposition of enamel and dentine. The upper incisor measures 3.6 mm. in length and the lower 5 mm. The position of the apex of the tooth is in close relation to the oral epithelium (fig. 18). A thickened mass of epithelium, partly a derivative of the original stalk of the enamel-organ, and partly an ingrowth from the

Fig. 18 Longitudinal section of upper incisor of 4-day albino rat, showing the increased curvature of the outline of the tooth and the relation of the apex of the tooth to the ingrowth of the oral epithelium. $\times 16$.

Fig. 19 Longitudinal section through basal end of labial side of enamel-organ of 4-day albino rat showing the region of the thickened margin. a, margin composed of mass of proliferating cells; b, region where three layers are seen; c, region where stratum intermedium becomes differentiated from rest of middle layer. Anterior to the region of this figure the epithelial papillae appear and the ameloblasts begin to form enamel. $\times 80$.



18



19

surface epithelium surrounding the tip of the tooth, is a preparation for the eruption of the tooth, and will serve as a resistant ring of tissue through which the tooth will be pushed at eruption. It may be looked upon as a protective device, to prevent adjacent tissues from being carried out by the erupting tooth.

The typical enamel-organ seen on the labial side does not cover the apex, for the tall columnar cells give place here, first to cubical and then to flat squamous epithelial cells, which form but a part of the thick mass of stratified epithelium, constituting the epithelial sheath over the end of the dentine. The other layers of the functioning enamel-organ also lose their identity at the region where the ameloblasts cease to have their characteristic elongated form. As maintained by von Brunn ('87) and Sachse ('94), there is no enamel apparent over the dentine at the apex of the tooth.

The cells representing the enamel-organ on the lingual side can be traced forward for a short distance as a two-layered stratum. These cells are flattened, with oval nuclei. Beyond this point only a single regular row of cells is apparent, and about half way along the length of the tooth-structure, even this ceases to be definite, and apparently here the mesenchymal cells of the peridental tissues have grown between and scattered these cells. As a result of this activity of the mesenchymal cells in this region, the enamel-organ now ceases to exist as a complete conical investment of the tooth. Approaching the apex of the tooth on the lingual side, one finds the prolongation of the epithelial sheath as a thin layer of flattened cells which thickens as it passes forwards into the epithelial sheath.

The basal formative end of the enamel-organ consists of a thickened band of tissue, as shown in figure 18, and under higher magnification in figure 19. This end is thicker on the labial side than elsewhere and it curves inwards, as seen in longitudinal sections, thus considerably diminishing the diameter of the entrance to the pulp-chamber. The extremity of this mass of tissue (fig. 19, a), constitutes a common origin for the several layers of the enamel-organ and contains many dividing cells. A short distance (0.1 to 0.2 mm.) from the extremity (fig. 19, b)

the cells form three layers, inner, middle and outer. The inner and outer layers, made up of columnar elements, stain more darkly than the middle layer, and the inner is thicker than the outer. The middle layer consists of elongated cells with oval nuclei, arranged for the most part with their long axes parallel to the surface of the enamel-organ. Frequent mitoses are also to be seen here, especially in the inner layer.

In the region about 0.6 mm. anterior to this (fig. 19, c), where enamel formation has not yet begun, the innermost layer shows a single row of distinct tall columnar cells, the ameloblasts. The middle layer now shows two subdivisions (a) two or three layers of compacted flattened cells lying against the ameloblasts, and composing the stratum intermedium, and (b) a somewhat thicker stratum, lightly staining, of more loosely arranged cells, constituting the enamel pulp. The outermost layer is a single row of cubical cells, which form a straight continuous surface for the enamel-organ. Beyond this layer and in contact with it are numerous small blood-vessels. Passing still farther forwards, the outermost layer becomes more sinuous in outline, and blood-vessels occupy the depressions between the elevations. This arrangement shows the beginning formation of the typical epithelial papillae.

Seven days old

At 7 days the tip of the tooth is in the oral epithelium (fig. 20), and ready for eruption, being separated from the outside by only a thin layer of superficial cornified epithelium. The epithelial tissues immediately about the apex of the tooth show the appearance of pressure atrophy. The cell boundaries are more indistinct than elsewhere, the tissue takes the acid stain deeply, and there is increased granularity—evidently degenerative effects due to the pressure of the advancing tooth.

In the upper jaw, the basal end of the tooth in its backward growth has reached the region of the maxilla, into which it continues to grow, pushing before it a little pocket of thin bone. The average length of the upper teeth at this age is 5 mm., and of the lower teeth, 7 to 8 mm. Their pointed apices, and their comparatively slight curvature are shown in figure 24.



Fig. 20 Osteodentine of apex of tooth of 7-day albino rat imbedded in the surface epithelium, showing cells in the lacunae in the matrix. $\times 175$.

ERUPTION OF TEETH

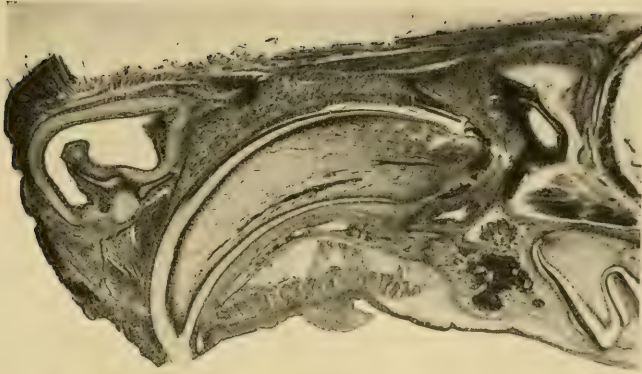
Eight to ten days

During the process of eruption (fig. 21), the tooth and its formative organs gradually move forward as a whole, and the apex of the dentine forming the anterior end of the tooth pierces the surface epithelium. This procedure is accompanied by new changes in the tooth-forming organs. For while the same process of cell-division continues at the basal end of the dental papilla and enamel-organ, these structures are subjected to

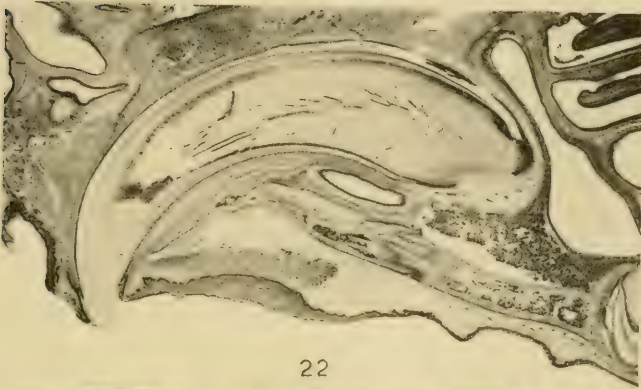
Fig. 21 Longitudinal section of the upper tooth of an 8-day albino rat, showing the apex of the tooth piercing the surface epithelium. $\times 10$.

Fig. 22 Longitudinal section of the upper tooth of a 12-day albino rat, showing the increased size and curvature of the tooth, the basal end directed more towards the palatal surface and the progression of the apex of the tooth through the epithelium. $\times 10$.

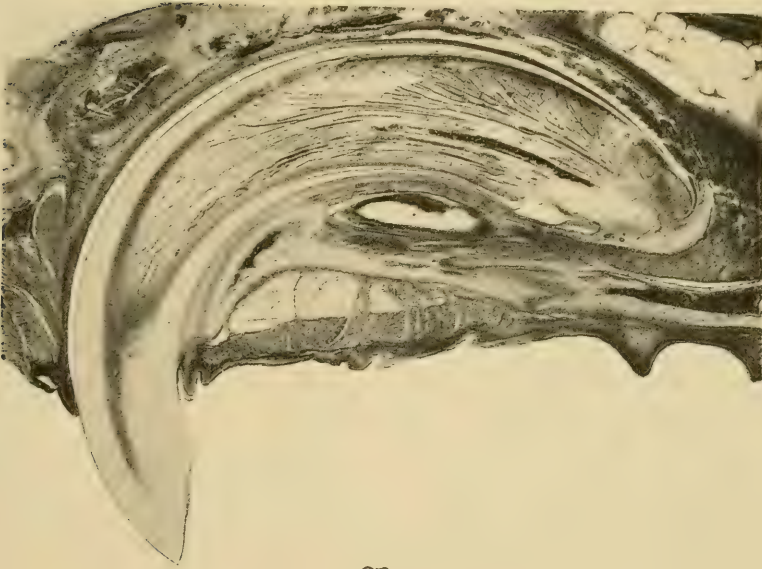
Fig. 23 Longitudinal section of the upper incisor of a 26-day albino rat, showing the well-established occlusal surface, the approximation of the basal end towards the palatal surface, abundant blood-vessels in the pulp, and the position of the granular osteodentine filling in apex of the pulp chamber. $\times 10$.



21



22



23

new conditions at the erupting end of the tooth. Before detailing these changes, it may be advisable to state, in a general way, the changing circumstances attendant upon eruption. Up to this time the anterior end of the tooth has been nearly stationary, but there has been continued growth backward of the posterior extremity. At this time the rate of progression forward is greatly increased, and the rate of progression backward much reduced. As suggested before, the process of eruption may depend largely upon the fact of increasing calcification in the bones, rendering them more resistant to the backward growth of the developing tooth. Whatever may be the causal factors, from now on the tooth continues to grow out at a regular rate, through the development of new cells at the basal end of the formative organs, these cells in turn giving rise to the hard parts of the tooth. Within a few days after eruption, the use of the tooth involves the process of attrition by which, in spite of the regular rate of growth, the exposed length is kept nearly constant for any age.

It is generally agreed that, by reason of the protoplasmic processes which extend into enamel and dentine from ameloblasts and odontoblasts respectively, these cells must be carried along with the tooth as it moves. Thus, as there is constantly a regeneration of these cells at the basal end of the tooth, there must be an opposite process of some nature by which these cells are eventually lost at the apical end, when carried thither by the outward progress of the tooth. First we may follow the history of the ameloblasts in this locality. Before eruption, the enamel-organ is continuous with the stratified epithelium forming the sheath around the gingival margin, and this relation continues at and after eruption. As the tooth moves forward during eruption the ameloblasts must move along with it and, when those at the anterior end approach the gingival margin, they must either be held there, or be carried out on the enamel until detached. On examining longitudinal sections at 12 days (fig. 22) it is seen that the ameloblasts, as they approach the gingival margin, become shorter and shorter, until, beneath the thickened sheath of epithelium

forming the gingival margin they acquire a flattened form. As a continuation of these flattened cells next the tooth is seen, extending out into the space between the erupted tip of the tooth and the epithelial gingival margin, a thin layer of tissue, which must be looked upon as the portion of the enamel-organ which has been carried out during eruption. At later stages this same appearance occurs—a thin layer of flattened cells continuous with the enamel-organ lying in the space between the tooth and the epithelium of the gingival border. It may be that some of the cells are added to the epithelium of the gingival margin, but the majority appear to be continually carried out, and eventually detached.

The mesenchymal tissues of the pulp at the anterior end are little affected by the mere act of eruption and not until some days later when attrition begins, do we see definite changes. At eruption the anterior conical extremity of the tooth is formed of osteodentine, containing within its matrix the remains of scattered cells and blood-vessels. Immediately posterior to this begins the true fine-tubed dentine with a central pulp-chamber. The cells at the anterior end of the pulp-chamber are irregularly arranged, but following backwards one soon sees the odontoblasts in parallel arrangement at the periphery of the chamber. At 10 days, when the apex of the tooth has pierced the epithelium and is easily seen from the outside, the measurements of the upper and lower teeth are 7 and 11 mm. respectively. At 12 days, they have increased to 7.5 and 11.8 mm.

CHANGES IN APEX OF TOOTH BY USE

Already at 12 days, when one examines the exposed ends of the teeth, they show little pits, which have been caused by the pressure of the opposing teeth. At 14 days, the ends are flattened, and at 16 days, because of the increased obliquity of this flattened surface due to the wearing away of the lingual side of the dentine, they are acquiring a cutting edge. The length of these occlusal surfaces continues to increase so that by 19 or 21 days (fig. 24), they have nearly the appearance typically

seen in the fully developed teeth. The osteodentine of the tip of the tooth is softer than is true dentine, for when the young tooth is dried this end shrivels and darkens in color. This cap of osteodentine on the end of the tooth may be useful, as suggested by Sachse ('94), because of its softness, in allowing the early formation of the functional occlusal surface. When this soft substance begins to wear away the tissues of the pulp would soon become exposed were there not a provision for the filling in of the apex of the pulp-chamber. This is effected by the formation of an irregular hard matrix, which may also be called osteodentine, within the extremity of the pulp-chamber. As



Fig. 24 Isolated upper and lower incisors of several ages of young albino rats. The pointed shape just before eruption is seen at 7 days. At 12 days, there is yet very slight change in the apices. At 21 days the occlusal surfaces are concave, and at 26 days they have nearly the typical mature appearance. $\times 2$.

the outer surface of the tooth wears away, this formation is constantly taking place a short distance from the occlusal surface.

Thus in examining a longitudinal section of the tooth at an age when the process of attrition has begun, and the typical occlusal surface has been formed (e.g., 26 days, fig. 23), we find this form of secondary dentine or osteodentine filling in the distal extremity of the pulp-chamber. As one approaches the anterior end of the pulp-chamber, the pulp becomes more and more restricted and the blood-vessels appear congested. Proceeding distally, the irregular matrix formation is seen between the cells and blood-vessels and finally near the occlusal worn surface is a granular mass of osteodentine with no circulating blood in it, but spaces are still seen containing the remains of the pulp elements. Here the living elements have disappeared,

but by staining (e.g., with acid fuchsin), the remains of these may be made out in less calcified spots in the matrix. Evidently the odontoblasts and other tissues of the pulp which move with the dentine, become more and more compressed at the narrowing apex of the pulp-chamber, and finally there is this irregular deposit of secondary dentine between them, which serves to obliterate the pulp-chamber. As the tooth moves out, this process is constantly going on, just in advance of the occlusal surface, and keeps pace with the process of attrition.

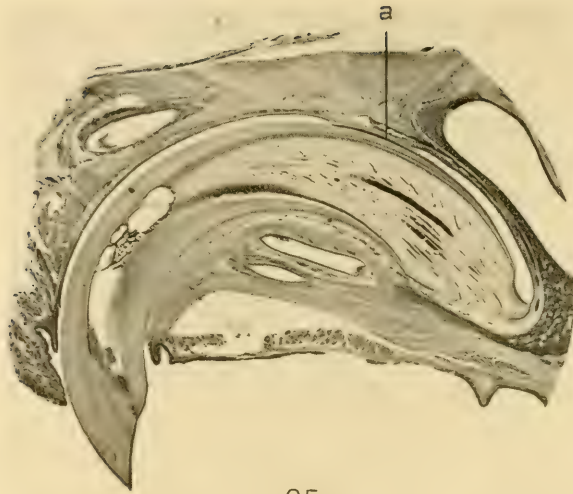
It is interesting to note the rate at which the teeth are increased in length during their formative period and prior to attrition.

	<i>Upper</i> <i>mm.</i>	<i>Lower</i> <i>mm.</i>
1 day old.....	2.3	3
4 days old.....	3.6	5
7 days old.....	5	7-8
10 days old.....	7	11
Average growth 0.52 mm. and 0.88 mm per day		

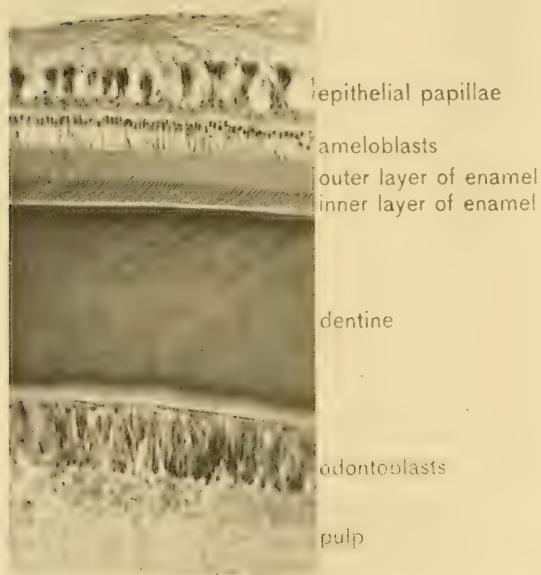
As will be seen later, this exceeds the rate at which the mature tooth continues to grow out.

DESCRIPTION OF MATURE TOOTH AND TOOTH-FORMING ORGANS, IN FIVE-MONTH ANIMAL

In the mature tooth, the general relations are shown in figure 25, made from a photograph of a decalcified section of the upper tooth of a 5-month animal. The regular curved outline is seen, with the greater proportion of the length imbedded within the jaw, and only a small part projecting. The formative end lies within an investment of bone belonging to the maxilla. At this end the dentine is very thin and the pulp greatest in amount. As one goes forward, the dentine increases regularly in thickness while the pulp-chamber becomes smaller and smaller. The vacuolated appearance at the anterior end of the chamber is due to shrinkage of the pulp tissue during fixation. The enamel has been lost in the process of decalcification except over the basal third. Numerous blood-vessels are seen within the pulp.



25



26

Fig. 25 Longitudinal section of upper incisor of a 5-month albino rat. The letter *a* shows where the next illustration (fig. 26) is taken. $\times 6$.

Fig. 26 Small portion of the preceding, more highly magnified, to show the enamel-organ and the enamel, and the odontoblasts and dentine. $\times 135$.

The enamel-organ is continuous over the convex labial surface of the imbedded portion of the tooth but is restricted to the most posterior region of the other surfaces, extending only 1 mm. forward from the basal margin. The enamel-organ differs in its structure in three regions of the labial side, and may be described separately in these three parts: (1) at the basal formative end, (2) near the gingival margin and (3) in the long intervening region. In (1) the enamel-organ is being constantly regenerated by the addition and growth of new cells. In (2) the enamel-organ is undergoing a retrograde process, while (3) represents the region where the enamel-organ is at its highest functional development, although its activity in increasing the thickness of the enamel is restricted, as noted before, to the basal third or less in the upper and to the basal half in the lower tooth.

Considering first the region (3), as shown in figure 26, the enamel-organ is conspicuous by reason of its tall ameloblasts and the high, narrow papillae. The enamel-organ is described in three layers—inner, middle and outer. The inner layer consists of the ameloblasts, which measure about 40 μ in height, with nuclei situated towards the outer end of the cells. The middle is composed of two strata (a) stratum intermedium, and (b) enamel pulp. The stratum intermedium is formed of 1 or 2 rows of fairly regular cuboidal cells resting upon the outer ends of the ameloblasts, but the enamel pulp is not now recognizable as a distinct layer and exists principally within the papillae. The outer layer of the enamel-organ consisted originally of a single layer of cells, but these are no longer regular in form or arrangement. Together with the remains of the enamel pulp, the outer layer forms the papillary elevations, 60 to 70 μ in height. These papillae are surrounded by an abundant capillary blood supply for the nourishment of the cells engaged in the formation of the enamel, and the purpose of the elevations is apparently to increase the surface area through which absorption may take place from the blood-stream.

The enamel is in two layers (fig. 26), the rods while traversing the inner layer being very distinct, and inclining towards

the apex of the tooth at an angle of from 50 to 54° with the dentine surface. The continuations of these rods in the outer layer are not so distinctly seen, but the inclination, as made out in thin sections, is still greater towards the apex, forming an angle of from 20 to 25° with the plane of the surface of the dentine. As noted before in the study of enamel, the rods in the inner layer, when observed in cross-sections of the tooth, decussate at an angle of from 70 to 90° , but when they reach the outer layer all run parallel. The fact that the rods run in these various directions seems incontrovertible, but in the light of our present knowledge of enamel formation it is difficult to understand how this condition is arrived at. If each ameloblast is responsible for an enamel-rod, then it follows that because the alternate layers of rods are oblique to one another, the ameloblasts responsible for these series of rods must have changed their relative positions during the process of formation of these rods. No such phenomenon has been observed, or even suggested. The other possibility is that the matrix of the rods is formed in a regular manner, but that afterwards, before calcification is complete, the rods become re-arranged owing to pressure strains.

The plane of direction of the rods is suggestive of the importance of the enamel-organ in the persistent growth. For always the general plane of the rods, as they leave the enamel-dentine junction, is towards the outer end of the tooth, as if the ameloblasts, while engaged in enamel-formation were always held back by the enamel, in which their processes were imbedded.

The basal formative end of the enamel-organ (region 1) in the adult animal corresponds very closely in structure with what has already been described for earlier ages, e.g., 4 days. This is the region where the enamel-organ is constantly being renewed, and it retains the same embryonal character at all stages of development.

At the anterior end where the enamel-organ is continuous with the surface epithelium (region 2), a gradual transition oc-

curs between the typical enamel-organ and the stratified squamous epithelium (fig. 27). As one follows the innermost layer of the enamel-organ forward, the cells become shorter, until they are cubical and finally flattened in shape. Here the other layers also lose their regular arrangement, and form, with the preceding, a thin layer of stratified cells. This layer can be followed directly into contact with the epithelium of the gingivus.

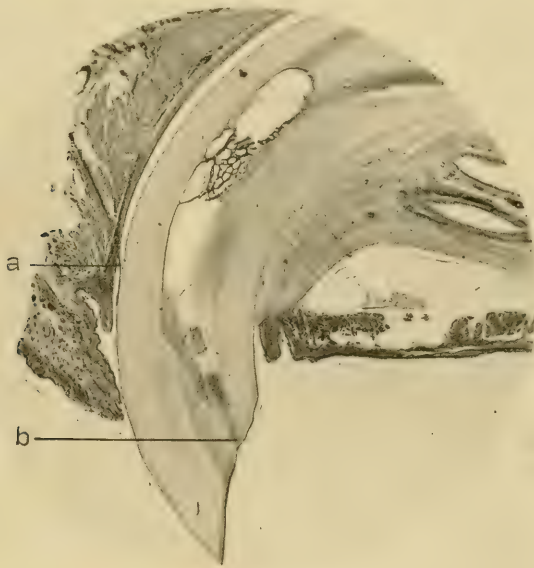


Fig. 27 Longitudinal section of the apex of the tooth of a 5-month albino rat, showing at *a* the position of the outward prolongation of the remains of the enamel-organ, and at *b* the more granular osteodentine filling in the apex of the pulp chamber. $\times 10$.

The cells, however, do not lose their identity in the surface epithelium but remain separate as a thin layer lying against the enamel (fig. 27, *a*). This thin layer of epithelium, therefore, represents the ultimate fate of the enamel-organ after it has completed its functional activity. It is being continually pushed out and its most anterior part must be continually being lost.

RATE OF GROWTH OF THE INCISOR TEETH

Two methods were used for determining the rate of growth of the incisor teeth (a) cutting off one or more teeth at the gingival margin and (b) making marks upon the enamel. The results here given are based on the latter method, as giving more nearly the normal rate of growth. By means of a dental engine, the animal always having been anesthetized, a fine transverse notch was made on the enamel of the incisors a short distance from the gingival margin. The interval between this mark and the tip of the tooth was then measured. At the end of about a week the distance between these two limits was again taken, and the difference between the two measurements showed the amount of wearing away. Two series of experiments were made by this method upon adult animals. In the first series six animals were used and in the second four animals, and measurements were made for several consecutive weeks. The longest period that one individual was studied was six weeks.

The two series gave very similar results. The lower tooth was always found to grow more rapidly than the upper. The upper tooth averaged 0.31 to 0.32 mm. per day, or 2.2 mm. per week, while the lower tooth averaged 0.4 mm. per day, or 2.8 mm. per week. No doubt there are many variations of the rate of growth under different circumstances, so that these figures must be taken as representing the average rate under one particular set of conditions. The food of these animals was the mixed diet now in use in the rat colony of The Wistar Institute. A short series of trials was made with animals kept in a large glass jar and given only soft food. In these animals the rate of wearing away corresponded very closely to that seen in animals which have also hard food and have the opportunity of gnawing. In these, therefore, the interaction of the opposing teeth must have caused the attrition.

For assistance with these experiments we wish to thank Dr. Stotsenburg, who greatly aided us in carrying out our observations.

It is interesting to compare these results with those obtained on the rabbit—the only other rodent which has been carefully studied in respect to the growth of its incisors. MacGillavry ('75), using a young adult rabbit, made marks upon its lower incisors 2.5 mm. and 3 mm. from the tip. After five to seven days the marks had disappeared. Evidently the rate of growth was about 2.5 to 3 mm. per week. Noë ('02) used a rabbit which happened to possess overgrown teeth. The animal accidentally broke off the lower incisors in the bars of its cage, and Noë made observations upon the rate of their growing out. This he found to be .615 mm. per day, or 4.3 mm. per week. This is larger than MacGillavry's results and may have been due to the unopposed growth and to the other abnormal conditions which may have been present in the formative organs.

Using MacGillavry's figures for comparison, it would seem that the lower teeth of the albino rat and of the rabbit grow out at about the same rate.

OVERGROWTH OF INCISORS

Examples of overgrowth of the incisors of rodents, especially in rabbits and hares, which were hunted as food, must have been observed from early times. In the older literature, they are referred to principally as curiosities, which have excited the interest of whoever has found them. Later the causes of the malformations were also considered. Thus Jenyns ('29), to cite only one observer, found several examples in wild rabbits, and has given a good illustration of the curved aspect of the teeth. He also clearly states the several causes which, in his opinion, may give rise to the condition. In addition to the one usually accepted at his time—accidental breaking off of one tooth—he considered also as causes (a) too soft food, (b) morbid or too rapid secretion of the osseous matter of the teeth, and (c) dislocation of one of the condyles.

Wiedersheim ('02-'03) has reported a case occurring in a rat, where he found an associated assymetry of the cranium. He is in doubt as to which was cause and which was effect—the overgrowth of the teeth or the assymetry of the cranium.



Fig. 28 Cranium of albino rat, showing the overgrown upper incisors recurving to the left side. The left incisor passes to the outer side of the skull, while the apex of the right incisor has penetrated the bone of the maxilla in the region of the basal end of the left incisor. $\times 1$.

Fig. 29 Cranium of the same albino rat shown in the preceding figure, viewed from the right side. It shows the overgrown lower incisors recurving to the right side, and the cavity which the right incisor has worn in the palate bone.

Figures 28 and 29 show a skull obtained some years ago from the rat colony of The Wistar Institute by Dr. Stotsenburg, and prepared in the Histological Laboratory by Miss E. F. Brooks. The upper teeth curved to the left side of the head and the lower to the right side. As seen in figure 29, the right lower has penetrated through the bone of the palate into the

nasal chamber, while the right upper (fig. 28) has recurved and grown into the maxilla.

In The Wistar Institute rat colony, at the time when the animals were fed on bread and milk, frequent examples of this and similar conditions were found, but now under a more varied mixed diet they practically never occur.

Beretta ('13) has recently made an analysis of these abnormalities and has classified them in three groups.

(1) Overgrowth of the upper and lower incisors through lack of an opposing tooth.

(2) Overgrowth of the incisors of the upper and lower jaws through deviation of the jaws.

(3) Prognathism of the lower jaw, and as a result, overgrowth of the incisor of the lower jaw.

In the present instance, diet seemed to be the controlling factor, probably by reason of its influence on the hardness of the bone of the alveoli from which the teeth grew out.

SUMMARY

The rate of growth of the upper and lower incisor teeth of *Mus norvegicus albinus*, in the mature animal, averages 2.2 and 2.8 mm. per week, or 12.5 cm. and 14.5 cm. per year, respectively.

Growth is due primarily to the proliferation and growth of cells at the basal end of the enamel-organ, where new enamel-forming cells arise, and at the basal end of the dental papilla where new dentine-forming cells develop.

The enamel-organ of the adult forms a narrow circular band around the basal end of the tooth, and extends forward from this on the labial side only. It coincides in its lateral boundaries with the enamel, and extends along the entire imbedded portion of the tooth. Anteriorly, it comes in contact with the epithelium of the gingival margin, and is carried out continually as a narrow band of cells lying on the enamel, between the latter and the gingival epithelial tissue.

The first indication of the anlage of the incisors appears in 14-day-old fetuses. In fetuses, 21 days of age (just before

birth), enamel and dentine formation is beginning. In animals 1 day old the upper and lower teeth measure 2.3 and 3 mm. At 8 to 10 days the teeth erupt, and at 10 days measure 7 and 11 mm. respectively. This period is therefore characterized by the rapid elongation of the teeth.

The process of attrition begins within a few days after eruption, so that by 19 or 21 days of age, the typical occlusal surface is formed. Up to the time of eruption the anterior end or apex of the tooth is immediately under the oral epithelium, while the basal or growing end is continually progressing posteriorly. After eruption, the basal end becomes nearly stationary in position, while the whole tooth structure is continually moving forward. The extra-gingival length of the tooth is kept constant, however, by the attrition of the occlusal surface, either through use in gnawing or by the action of the opposing teeth.

The histogenesis of the enamel-organ is practically completed by the 4th day after birth, although it does not attain its final relations to the tooth as a whole, until after eruption. In the 18-day fetus the enamel-organ is similar in all parts, and the cells of the inner layer measure the same, both lingually and labially. From this period forwards, however, the labial portion continues to progress towards its fully differentiated functional structure, while the lingual portion retrogresses, until at 4 days after birth the latter is disrupted, by the ingrowth of the surrounding connective tissue. Contrasting the cells of the inner layer—the potential ameloblasts—on the labial and lingual sides, they are practically the same in the 18-day fetus, but at 19 days they are found to measure 24 and 20 μ respectively. In the 21-day fetus, they measure 30 to 34 and 12 μ , and 1 day after birth the true ameloblasts on the labial side have increased to 40 μ , while the non-functional cells of the lingual side are only 10 μ in height. At 4 days, the latter cease to form a continuous layer, by reason of the dispersion of the cells by the surrounding connective tissue, except at the basal formative region.

Characteristic of the permanently-growing enamel-organ are the epithelial papillae, formed by the elevations of the outer

layer of the enamel-organ, and the cells of the enamel pulp. Between these elevations are numerous capillaries which insure a rich blood supply to the enamel-forming cells.

There are three layers in the functional enamel-organ—inner, middle and outer. The inner is constituted of the tall ameloblasts, and the middle is made up of two divisions, (a) stratum intermedium and (b) enamel pulp. The latter unites with the single layer of cuboidal cells which compose the outer layer, to form the epithelial papillae (fig. 26).

The apex of the primitive tooth is formed of a variety of secondary dentine—'osteodentine' of Tomes—which is softer than true dentine, and differs in its structural arrangement (fig. 20). After eruption, this terminal portion of osteodentine is soon worn away by attrition, and the typical occlusal surface is developed, as seen at 19 or 21 days. At 21 and 23 days the first two molars erupt in both upper and lower jaws, and from now on the animal is able to secure food for itself, and if necessary can maintain an independent existence.

As the tooth continues to be worn away there is a provision for the continual filling in of the apex of the pulp-chamber by the formation of what may also be called osteodentine. This is a form of secondary dentine, containing, when first formed, cells and blood-vessels. This is always at a little distance, about 1 mm., from the occlusal surface, but as any part of the tooth, in its outward progression, approaches the occlusal surface, the soft elements disappear within the osteodentine, and the latter forms a hard continuous surface with the adjoining true dentine. The position of this osteodentine is marked as a line on the occlusal surface of the teeth (fig. 5).

Prior to eruption there develops around the apex of the tooth, as it lies in contact with the surface epithelium, a thickened ring of stratified epithelium. This ring of tissue is pierced by the apex of the tooth at eruption, and would seem to have the function of serving as a resistant margin for the soft tissues, and of preventing other tissues being carried along with the erupting tooth.

The length of the teeth varies with the size of the cranium (table 1) so that the persistent growth is not only sufficient to offset the continual attrition, but also serves to keep the length of the teeth in a definite relation to the length of the skull, as the latter increases in size.

The lower tooth is always longer than the upper, and this difference manifests itself even in the anlagen of these structures in the 19-day fetus (figs. 11 and 12).

The contour of the enamel, as seen in cross-sections, is characteristically different in the upper and lower teeth (fig. 4).

The enamel of the tooth is composed of two layers which are different in appearance. The enamel rods run in two sets which decussate with each other in the inner or plexiform layer, but they change their direction as they continue into the outer layer, so that in it they are all parallel. In longitudinal sections, the general direction of the rods (fig. 26), is to incline towards the apex of the tooth, as they run from the enamel-dentine boundary to the outer surface of the enamel.

In conclusion, we wish to thank Professor Piersol for generous assistance in many ways, and Professor Donaldson for his constant interest in the study. We also wish to acknowledge the kind assistance of Mr. E. F. Faber in the preparation of the drawings.

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A PECULIAR STRUCTURE IN THE ELECTROPLAX OF THE STARGAZER, *ASTROSCOPUS GUTTATUS*

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THREE FIGURES

The purpose of this paper is to determine the function and composition of the peculiar pointed fibers and long pointed rods lying in the electric layer of the electroplaxes of the stargazer, *Astrosopus guttatus*.

Before proceeding with a discussion of these rods, a brief description of the electric organ of this fish (according to Dahlgren)¹ will be given.

The electric apparatus is composed of two organs, which form two vertical columns roughly oval in horizontal section, and placed behind and somewhat under each eye. Each organ extends from the peculiar bare spot on the top of the head down to the tissues which form the roof of the oval cavity; and is composed of about 200 thin layers of electric tissue, which extend horizontally all the way across the organ. These layers of tissue are flat, and always at the same distance from one another. Each layer contains about 20 electroplaxes, the outlines of which present a very irregular or scalloped appearance. The electric tissue in which the electroplaxes are imbedded is in appearance a jelly-like or mucous-like tissue, usually known as electric connective tissue, and which I have shown in the course of my work to be of the same composition as white fibrous connective tissue. The nerve and blood supply runs in the above tissue. The general form of a vertical section of an electroplax is shown by figure 1, which is a drawing of part of a section of a single electroplax.

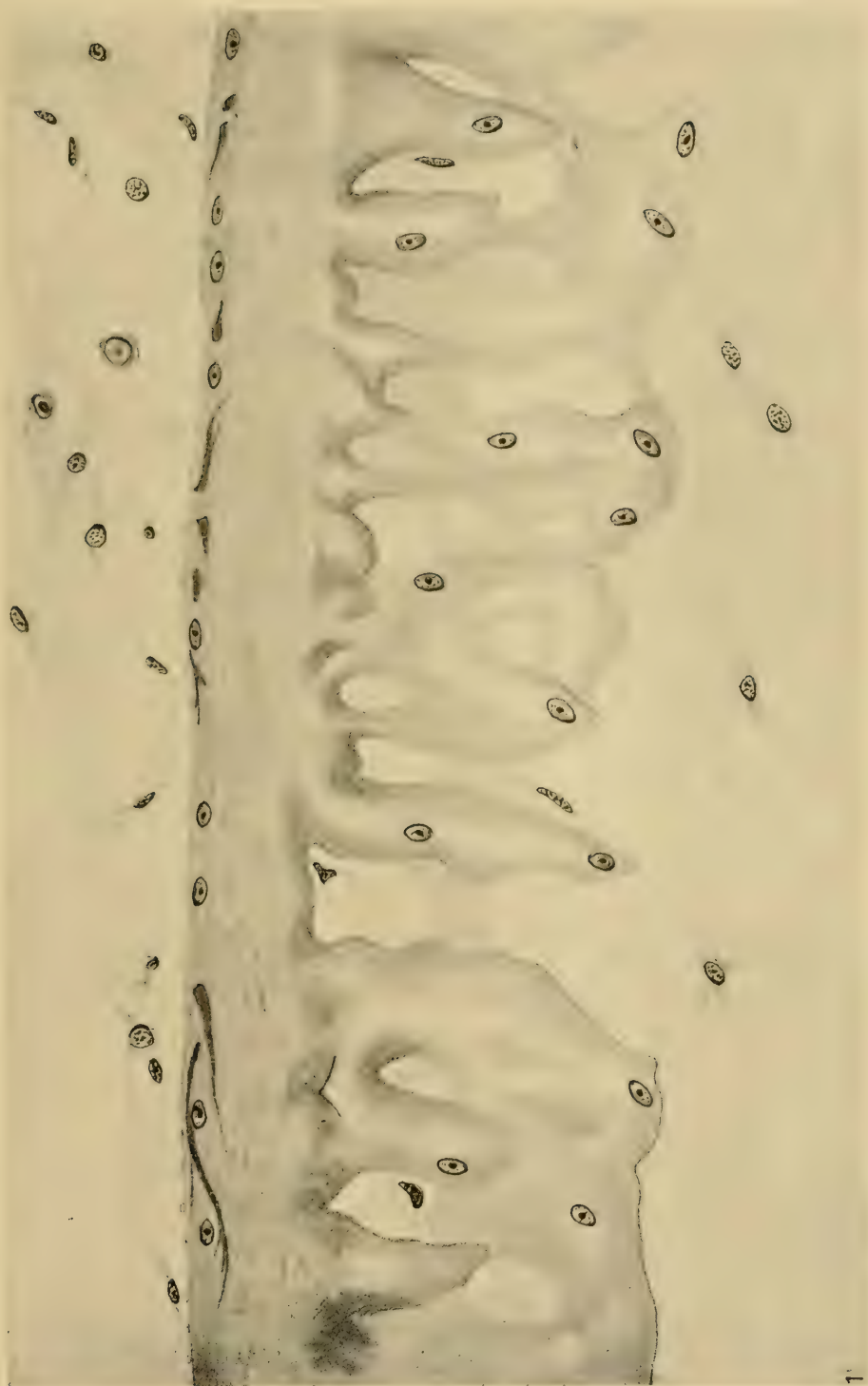
Each electroplax is composed of three principal layers, a nervous or electric layer which forms the upper surface, a

¹ Anat. Anz., Bd. 29, S. 387, 1906.

middle layer, and a lower or nutritive layer which along with the middle layer is evaginated into a large number of long papillae. All three layers are deeply marked with a dense series of fine striations, which are peculiar to the electroplaxes of several other fishes. The upper or electric surface is flat and smooth and receives the nerve endings. The current of electricity runs downward through the organ which produces it, and thus the nerve endings in accordance with Pacini's law are found on the negative pole of the electroplax.

Proceeding directly to the subject of this paper, we may say that one of the most interesting of the points noted in the electroplaxes, when properly fixed and stained with iron hematoxylin, is a series of rod-like or thread-like objects running horizontally in the electric layer, among, above and below the nuclei and without any apparent connection with them (figs. 1 and 2).² These rods are of various sizes and shapes, and in form are said to resemble the classic thunderbolts seen in the hand of representations of Jove. They usually taper slowly and branch extensively at one or both ends. Some of these branches sometimes seem to be mere lines, while others are wide and heavily pointed; at their other ends the rods are usually rounded; this latter appearance may be due, however, to the cut ends of the rods, for as noted above they sometimes branch at both ends. Some are short and heavy in appearance while others are long and thread-like. Peculiar looping, twisting, or knot-like bends are sometimes found at points on the longer rods. The outlines and contour of these rods are always smooth. Their size may vary from thick or thin rods of over 300 μ in length down to small ones that do not exceed 1 μ . In those electroplaxes where the rods are few they sometimes lie parallel and point in a definite parallel direction, while in others where the rods are very numerous they do not seem to have any definite arrangement. In this latter condition the rods present a very wavy appearance. Their form may be seen in figures 1 and 2, which are drawings of the electric layer of an electroplax when

² All the figures are drawings of sections of electroplaxes of *Astroscopus guttatus*.



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Fig. 1 Vertical section of part of an electroplax. The whole electroplax as well as the electric connective tissue is shown; rods are seen lying in the electric layer. $\times 1200$.

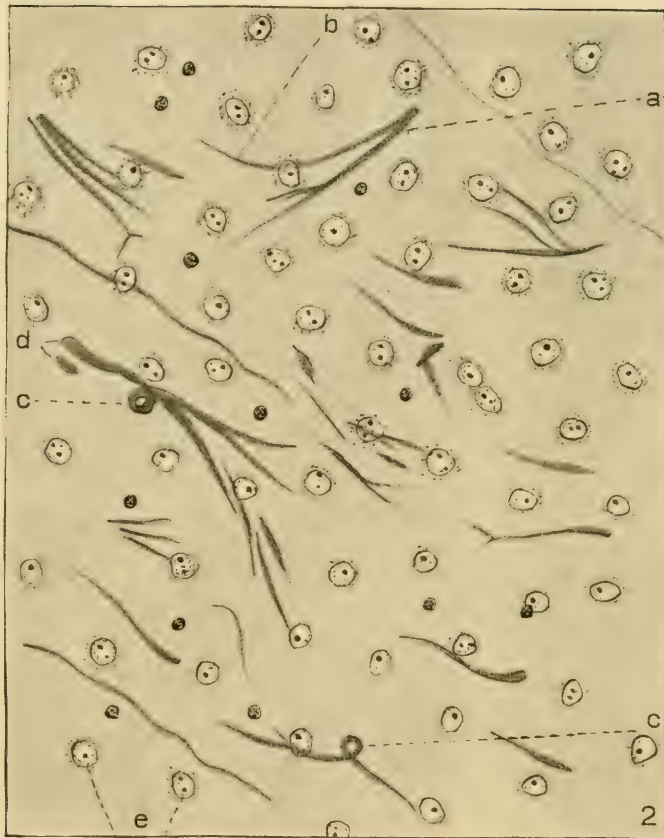


Fig. 2 Horizontal section through an electrophorus. Only the electric layer is seen; *a*, an extensively branching rod; *b*, fine branches of the above rod; *c*, a large characteristic loop in a rod; *d*, a cut end of a rod; *e*, nuclei of the electric layer. $\times 1200$.

stained with iron hematoxylin. These drawings show the electric layer in which the rods are found in horizontal section (fig. 2) and in vertical section (fig. 1).

The purpose, function, and chemical composition of these rods have been previously unknown to histologists. In order to determine anything in respect to their function or purpose, a knowledge as to the class of organic substance to which they belong, whether muscle, connective tissue, nervous, or chitinous,

and also a rough knowledge of their chemical composition is imperative. The contour and form of these rods as they appear under the microscope resemble both smooth muscle fibers and fibers of elastic connective tissue. The belief that the function of these rods was somewhat of the nature of support for the delicate substance of the electroplax, and the fact that their form resembled connective tissue fibers led the writer to take for one of his first hypotheses, that they were of some form of connective tissue, and to perform accordingly the following series of experiments. As the most logical and best way for determining the kind of connective tissue, if any, of which the rods might be composed, a number of stains used by other investigators to identify similar substances were applied and the results noted. Controls were used on known tissues.

Before taking up the connective tissue stains, however, a description of the results from the iron hematoxylin staining is now noteworthy; the material used being fixed in pure corrosive sublimate.

The jelly connective tissue stained a very light gray. The nutritive, striated, and electric layers stained a much darker gray. The nuclei in all the layers stained somewhat black. The pointed fibers, and rods found in the electric layer stained a deep black, thus being clearly differentiated from the surrounding cytoplasm. In some electroplaxes they were very numerous, while in others the number was rather small.

The connective tissue stains applied as follows:

(1) Mallory's connective tissue stain, using the modification given in Lewis' "Text-book of histology." White fibrous connective tissue should stain blue in this medium.

Paraffin sections of the electroplaxes fixed in corrosive sublimate were stained for 10 to 12 minutes in a 1 per cent aqueous solution of acid fuchsin. They were then transferred directly to a stain consisting of 0.05 grain of aniline blue (soluble in water) and 0.2 grain of orange G dissolved in 100 cc. of a 110 per cent aqueous solution of phospho-molybdic acid. In this they remained from 2 to 3 minutes. They were then rinsed in distilled water, dehydrated rapidly, cleared and mounted.

A description of an electropilax as seen under the 2 mm. oil immersion lens is as follows: The white fibrous or electric connective tissue stained a light blue or purple. The electric nutritive and middle layers or the electropilax proper stained a reddish purple, and the nuclei as a whole in all of the layers, stained somewhat lighter than their surrounding cytoplasm.

The peculiar rods and fibers stained a deep red, and were thus clearly differentiated from the other elements of the electropilax. They were very numerous and as noted above their outlines were always smooth. Blood corpuscles lying in the jelly connective tissue, of which there were only a few, stained a brilliant red, much the same as the rods.

The white fibrous or jelly electric connective tissue (between the electropilaxes), as noted above, stained a light blue. The pointed fibers and rods stained a deep red. This would indicate therefore, that these rods are not composed of white fibrous connective tissue. It would seem also that they are not muscle for they stained a different shade of color from the rest of the electropilaxes, which I have found in the course of my work to stain much the same as muscle.

(2) Van Gieson's connective tissue stain, in which white fibrous connective tissue should stain red:

Paraffin sections of the electropilaxes fixed in corrosive sublimate were stained for 4 to 5 minutes in a 1 per cent aqueous solution of hematoxylin. They were rinsed in distilled water and transferred to a stain consisting of a saturated aqueous solution of picric acid containing 20 per cent acid-fuchsin. They remained in here 15 to 20 minutes and were then rinsed, cleared, and mounted. The white fibrous connective tissue layer stained pink. The three layers of the electropilax stained brown. The nuclei and rods stained the same color, that is, brown; and, while the nuclei could be seen with difficulty, the rods were scarcely visible owing probably to their similar refractive index. The fact therefore that the rods did not stain the same color as the white fibrous connective tissue, which stained pink, indicates again that they are not composed of white fibrous connective tissue. By Van Gieson's stain, therefore, I have

confirmed the evidence as presented by Mallory's stain in respect to the composition of the rods; that is, they are not white fibrous connective tissue.

I now undertook to apply stains which were tests for elastic fibers, and the first under this head is Weigert's resorcin-fuchsin stain in which connective tissue according to Weigert and other writers stains dark blue. The stain was made up as follows:

One per cent of basic fuchsin and 2 per cent of resorcin were dissolved in water; 50 cc. of the solution were raised to the boiling point, and 25 cc. of liquor ferri sesquichlorate P.G. were added and the whole boiled with stirring from 3 to 5 minutes; a precipitate was formed. After cooling, the liquid was filtered, and the precipitate which remained on the filter was boiled with 50 cc. of 95 per cent alcohol. It was then allowed to cool, filtered and the filtrate made up to 50 cc. with alcohol, and 1 cc. of hydrochloric acid added.

Paraffin sections of the electroplaxes fixed in corrosive sublimate were stained for 6 hours in the above alcoholic solution of the precipitate. They were then washed in 95 per cent alcohol, dehydrated quickly, cleared and mounted. A description of the results are as follows: The electroplax proper, with its three layers, did not take the stain at all. The jelly electric tissue around the electroplax stained a brilliant blue. The rods contained in the electric layer of the electroplax were invisible. The fact therefore that the rods did not take the stain at all shows they are not composed of elastic connective tissue. The control used in this case for elastic tissue was a section of the ligamentum nuchae of a horse, which, when put in the stain for exactly the same time as the electroplax, came out a deep blue. The ligamentum nuchae was chosen as a control since it is, perhaps, the best known and best representative of elastic tissue found in the animal kingdom.

Not desiring to rely solely on the above stain to prove that the rods are not composed of elastic tissue, the electroplaxes were treated with the digesting fluid, pepsin, and the results noted. Vertical sections of electroplaxes, having been freed from paraffin, were put for 3 minutes in a very weak solution

of pepsin in 0.2 per cent HCL (the concentration being 0.5 gram of commercial pepsin to 100 cc. of 0.2 per cent aqueous HCL). The electroplaxes were then treated with the following stains: (1) Iron hematoxylin. The result was that all the rods and part of the nuclei, and less dense portions of the electroplax had been digested. (2) Mallory's connective tissue stain was applied, the electroplaxes being stained according to the directions given in the first part of the paper. The results were the same as with the iron hematoxylin, namely, the less dense portions of the electroplaxes and the rods had disappeared. The fact therefore that these rods were digested in 3 minutes by a weak solution of pepsin discredits absolutely the hypothesis that the rods are composed of elastic connective tissue, for elastic fibers are digested only very slowly by pepsin, the time required being several hours; the control in this case being the same as that noted above—the ligamentum nuchae of a horse.

Having shown that the rods are not composed of connective tissue of any sort, the next most logical hypothesis was that they were of some form of keratin, chitin, or chondrin, and the following test was performed accordingly.

Vertical sections of the electroplaxes of *Astrosopus*, after the paraffin had been removed, were put for 24 hours in a 72 per cent solution of hydrochloric acid. The acid was then washed out and the sections stained with iron hematoxylin and then examined with the microscope. The rods could not be found, but hollow spaces corresponding to the shapes of the rods were found in the electric layer of the electroplax. Figure 3, a drawing of a slide after treatment with hydrochloric acid, shows these spaces clearly. The spaces are seen to be a little wider and larger than the rods, showing that the rods must have swelled to a certain extent before being dissolved by the acid. This latter statement was also confirmed by treating the rods with the acid for only a short time, and then examining with the microscope; the result being that the rods had swelled to a considerable amount. The other elements of the electroplax, nuclei, etc., in their general form remained intact. There-

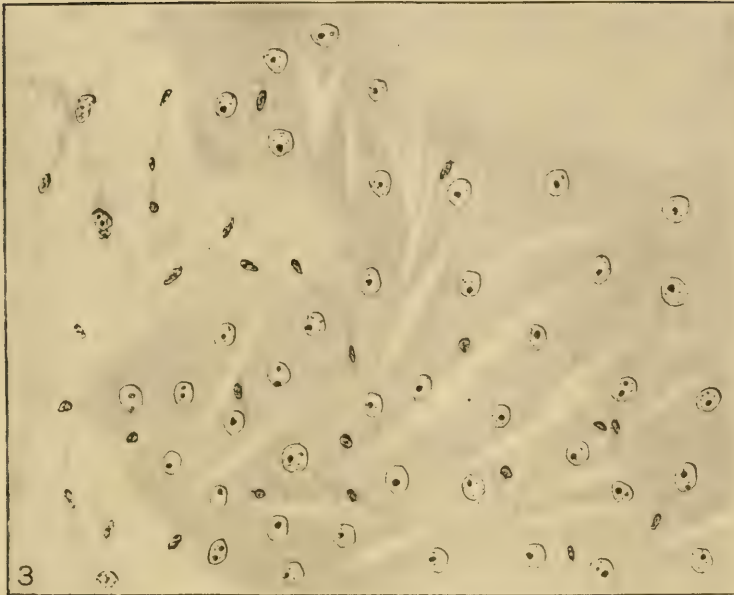


Fig. 3 Oblique section through an electroplax treated with 73 per cent hydrochloric acid. The portion of the electric layer shows the electric nuclei, and also the spaces which were occupied by the rods before they were dissolved. The spaces have a greater diameter than that of the rods because the rods swelled before being dissolved. $\times 1200$.

fore this indicates clearly that the rods are not composed of chitin or keratin, for these substances are not attacked by hydrochloric acid of the above strength. To strengthen this statement we have only to quote the results from the pepsin treatment noted above, in which the rods were digested in 3 minutes. They could not therefore have been composed of chitin or keratin, for according to *Encycl. mikr. Technik*, these substances are not attacked at all by pepsin. From the above facts therefore we may with a good degree of certainty conclude that the rods are not composed of any kind of chitinous or keratinous substance. Having found that the rods do not consist of any kind of connective tissue or of chitin or keratin the only reasonable hypothesis left was that they were some form of muscle fiber, not having however the same chemical com-

position as the ordinary striated fiber, from which the electroplax is derived. Accordingly the following stains were applied:

(1) Van Gieson's picro-nigrosine, which stains muscle a yellowish-green and connective tissue blue, mixed as follows: To 45 cc. of saturated aqueous solution of picric acid, 5 cc. of 1 per cent aqueous solution of nigrosine were added, the whole mixed thoroughly. Paraffin sections of the electroplaxes (vertical) fixed in pure corrosive sublimate were stained for 12 hours in this mixture; then washed in picric alcohol, dehydrated rapidly, cleared and mounted. A description of an electroplax is as follows: The jelly electric tissue stained blue. The electroplax proper with its three layers stained a yellowish-green. The rods were just visible, and were stained the same color and to the same degree as the electroplax. The fact therefore that the rods stained the same color as the electroplax and as muscle shows that they are very probably composed of some muscle-like substance. It may be noted here that the electroplax proper always stains about the same as voluntary muscle, a control consisting of voluntary muscle having proved the truth of this statement several times. The reason for this is apparent when we recognize the fact that the electroplax is derived from a striated muscle fiber. This stain also confirms the results of Weigert's resorcin-fuchsin stain that the rods are not composed of any kind of connective tissue, and also indicates decidedly that the jelly electric tissue is white fibrous connective tissue. The controls used were the adductor muscle from an oyster, the ligamentum nuchae of a horse, and the white fibrous connective tissue in the umbilical cord of a sheep, all being stained exactly the same period of time as the electroplax.

(2) Van Gieson's picro-fuchsin, which stains muscle yellow and connective tissue red, was mixed as follows: To a saturated solution of picric acid were added a few drops of a saturated aqueous solution of acid fuchsin, until the mixture became a deep red. The stain was then ready for use. (It may be noted that, if too much acid fuchsin be added, muscle as well as connective tissue will stain *red*).

Paraffin sections of the electroplaxes (vertical) fixed in pure corrosive sublimate were stained for 6 hours in the above mixture. The results were as follows: The jelly connective tissue stained red, the electroplax proper with its three layers stained yellow. The rods were scarcely visible, staining the same color (yellow) as the electroplax. The fact that the rods stained yellow would indicate again that they are composed of muscle tissue, and this in connection with the picro-nigrosine stain in which the rods stained the same color as muscle gives us strong ground for believing that the rods are composed of muscle tissue; probably involuntary muscle fibers as the rods are not striated. The fact also that the jelly electric tissue stained red shows again that it must be of the same composition as ordinary white fibrous connective tissue. The controls were the same as those for Van Gieson's picro-nigrosine.

It may be objected that the rods did not stain the same color as the electroplax proper with Mallory's connective tissue stain. To explain this we may say that the rods stained a brilliant red, which is the color that muscle tissue should take in the above stain. The fact that only the electroplax stained a reddish-purple shows that the rods are more strictly composed of a muscle substance than the electroplax itself.

The only tissue now left of which the rods might be composed is nervous; that is, the rods might be nerve endings of some kind, which they resemble a little. In order to test this Paton's silver nitrate stain for demonstrating nerve fibers and endings was applied and the results carefully noted. The stain and fixation were as follows:

The electric organ was fixed in 10 per cent formalin solution, neutralized with magnesium carbonate. It was then cut in small strips about 4 mm. thick. These were washed with running tap water for 12 hours and then in three changes of distilled water for about 30 minutes. The tissue was then put in 1 per cent silver nitrate solution for 6 days *in the dark*. The tissue became a reddish-brown in color. (It may be noted here that if the tissue becomes a yellowish-brown the stain has not been applied correctly, and it is advisable to throw the whole speci-

men away.) It was then placed in a *freshly* prepared solution of silver nitrate, made as follows:

To 20 cc. of 1 per cent silver nitrate solution, two drops of a 40 per cent solution of caustic soda were added. A gray precipitate was formed. Twenty to thirty drops of strong ammonia were then added—just enough to dissolve the precipitate. The tissue was allowed to remain in this for 45 minutes; then washed in distilled water containing a few drops of glacial acetic acid (25 drops of acid to 100 cc. of water). It was left in this for about 10 to 15 minutes, until the reddish-brown color had changed to yellowish-brown. It was then washed in distilled water, and placed for 12 hours in 1 per cent hydroquinone containing 5 per cent neutral formal. It was again washed with distilled water, dehydrated, and imbedded in paraffin through chloroform. The tissue was then cut and sections mounted with balsam.

A description of a vertical section of an electroplax so stained is as follows: The electroplax proper stained a very light brown. The nuclei, staining a little deeper, could be clearly seen. The electric connective tissue did not stain at all, and was not even visible. The nerve endings, which were very abundant, stained dark brown or black, thus being clearly differentiated from the rest of the electroplax. The rods could not be found. If present, they must have stained light brown, the same color as the electroplax, for they certainly were not present as nerve endings, which as I noted above, stained a dark brown or black. This conclusion therefore is evident, that the rods are not nerve endings of any kind.

The fact therefore that these fibers and rods have been shown to be composed of a muscle-like substance brings up a new kind of muscle fiber to the attention of histologists. These fibers have not been shown to be contractile, are non-striated, dense and exist without any apparent connection with each other. In view of the above facts we cannot give to these elements of the electroplax any other function except that of support, and this will consequently have to suffice until some other investigator presents a better interpretation.

CHROMOSOME STUDIES

III. INEQUALITIES AND DEFICIENCIES IN HOMOLOGOUS CHROMOSOMES: THEIR BEARING UPON SYNAPSIS AND THE LOSS OF UNIT CHARACTERS

W. REES BREMNER ROBERTSON

FOURTEEN FIGURES (THREE PLATES)

In November, 1910, while preparing a larger paper upon the problem of synapsis in the germ cells of certain grasshoppers belonging to the subfamily Tettigidae (Robertson '15), I found some individuals in which there occurred a pair of unequal homologous chromosomes. I made a series of drawings of these pairs at the time, intending to incorporate them later into my larger paper. That paper is not yet completed, owing to the duties of teaching during the last year at Kansas University. The importance of these inequalities in members of a chromosome pair, however, seems to warrant publishing the results in brief form. In this account I wish to point especially to a possible relation between these unequal chromosomes and the behavior of certain unit characters in breeding; also to the bearing which the permanency of such chromosomes has upon the problem of parasynapsis.

In the Tettigidae, a subfamily of the short-horn grasshopper family Acrididae, I have found, for all the species of at least four different genera which I have examined, the number of chromosomes to be uniformly 14 in the female (figs. 1, 11) and 13 in the male (figs. 4, 9). I have also found all to have, with limited variation, among the autosome (ordinary chromosome) series, two extremely long pairs of chromosomes, the 7's and 6's (figs. 1, 3, 4), two intermediate pairs, the 4's and 3's (figs. 1-3) or 5's and 4's (figs. 9, 10), and two very small pairs, the 2's and 1's (figs. 1-3, Tettigidea) or the 3's and 2's (figs. 9-10, Acridium).

The sex chromosome—single in the male and paired in the female—may rank in size as No. 5 (*Tettigidea*), No. 3 (*Paratettix*) or No. 1 (*Acridium*), depending upon the genus, but this variation between different genera does not seem to be accompanied by any very considerable difference in the relative sizes of the ordinary chromosomes.

Of the constancy of these size relations, I am very certain, having examined a large number of individuals in each species. Being certain of the size relations, I was very ready to recognize any abnormal variations in this respect when they appeared. I found such variations in two genera: two cases in *Tettigidea parvipennis*, only one of which is given (figs. 4-8), and two in *Acridium granulatus* (figs. 11-13).

1. A DEFICIENT NO. 4 CHROMOSOME IN *TETTIGIDEA* *PARVIPENNIS*

To understand the abnormal chromosomes we must first examine the normal ones. Figures 1 to 3 are of cells from normal individuals. Figure 1 is from the wall of an egg tube. It shows 14 chromosomes, the number characteristic of the female. The chromosomes are numbered and paired according to size. Two of the chromosomes (nos. 1 and 2) have been drawn at one side for convenience. This figure is also typical for male 2x cells, with this exception, that the sex-chromosome, No. 5, is always unpaired in the male. Figures 2 and 3 are lateral views of the first maturation division in the male germ cells. These show the members of pairs about to separate from each other. The members are still in contact at the distal ends in most cases. The No. 7 pair in figure 2 has formed a cross and is still paired through a greater extent than in the other pairs. The sex chromosome ranks fifth in size in *Tettigidea* and may be seen passing undivided to one pole in these cells. Among the autosomes (ordinary chromosomes) of figures 2 and 3 there can be seen a very slight difference in size between the 1's and 2's, a considerable difference between the 3's and 4's, and a much greater difference between the 6's and 7's. We are concerned at present with the relative sizes of the 3's and 4's only.

Since the diameters of the chromosomes are uniform throughout the series in any given cell, the relative sizes can be learned best by measurement of lengths. My measurements have been made from drawings outlined by means of a camera lucida, the image being projected at the level of the base of the microscope. A 2 mm. Leitzoil-immersion objective and an $18 \times$ Zeiss compensating ocular were used for outlining. I feel reasonably certain that my drawings show approximately the correct relative sizes of the chromosomes in each cell.

The average length (doubled) of the No. 3 chromosomes in the figures ($\times 2600$) of six cells from two normal individuals of *Tettigidea parvipennis* is 15 mm. The average length of the No. 4 chromosomes under the same conditions in the same six cells is 17.05 mm. The ratio of the No. 3 to the No. 4 chromosomes is therefore as 1 to 1.14. This may be taken as approximately the normal size relation of the No. 3 and No. 4 chromosomes; that is, No. 4 is about one-seventh longer than No. 3.

In figures 4 to 8 are cells from a male individual of the same species, in which one member of the No. 4 pair of chromosomes (4-) is abnormally small. The average length of the No. 3 tetrad is here 13.1 mm. This is shorter than in the preceding case of six cells, but in this instance all chromosomes are affected similarly, the reduction in size being due probably to the cells having been in slightly different stages when killed. This does not affect the relative lengths of the chromosomes, however. The average (doubled) length of the larger (no. 4) diad (figs. 5-8) in the same cells is 15.5 mm. The ratio therefore of the No. 3 tetrad in these cells to the larger diad of the abnormal No. 4 tetrad, or to a No. 4 tetrad made up of two such diads, would be as 1 to 1.18; i.e., the ratio of the No. 3 chromosomes to the normal member of the No. 4 pair here is as 1 to 1.18. This is not far from the normal ratio, 1.14, and the difference is quite within the range of the probable error due to inaccurate measurement, etc. But the average (doubled) length of the smaller diad of the abnormal No. 4 tetrad is 12.7 mm., instead of 15.5 mm. The ratio therefore of the No. 3 tetrad to a tetrad made up of two such No. 4 diads would be as 1 to 0.97; i.e., the ratio of the

No. 3 chromosomes to the smaller No. 4 chromosome is as 1 to 0.97. This shows the smaller No. 4 diad to be even smaller than the No. 3 chromosomes. I believe that the larger diad of this abnormal No. 4 tetrad is the normal No. 4 chromosome, since its ratio, 1.18, is so near to the normal ratio, 1.14, and that the smaller diad is abnormal and has lost a part of its distal end; (my reason for thinking the distal end deficient will appear later). Its ratio, 0.97, instead of 1.14, gives it a shortage of 0.17, or nearly one-sixth of 1.14. It seems therefore to have lost one-sixth of its normal length.

The size of this deficient No. 4 chromosome is constant for this individual. All division figures which occurred in lateral view showed the members of this unequal pair in practically the same relative sizes. Not only the germ cells but also somatic cells (fat body) exhibited the same proportions. The fact that the size ratio is constant in all germ cells and even in body cells seems to point to its germinal origin; i.e., it must have been present in the fertilized egg from which this animal developed.

There is no constant relation between the members of this unequal pair of chromosomes and the sex chromosome, as the cells of the maturation division show (figs. 5, 6, 8), for the sex chromosome passes as often to the pole which receives the small No. 4 chromosome as to the pole which receives the large member.

Another very noticeable evidence of the abnormality and defectiveness of this No. 4 chromosome is shown in its manner of contact with its larger normal mate in the first maturation spindle (figs. 5, 6, 7 c). In these three figures its attachment is not strictly terminal, as it should be. This, it seems to me, is evidence of a deficiency at the distal end of this chromosome. This will be better understood if I describe briefly the method of chromosome pairing in the *Tettigidae* (Robertson '15). During the period of synapsis, which occurs in the early part of the growth period of the first spermatocyte, homologous chromosomes pair side to side (parasynapsis), as shown in figures A₁, A₂ (pl. 3). After this period of synapsis, during the growth period which follows, a separation of the pairing chromosomes takes

place. This separation begins at the proximal ends of the pair and gradually moves along toward the distal ends (figs. A_3 to A_5). By the proximal end I mean that from which the spindle fiber springs and that which travels in advance toward the pole. The opposite, blunt end is the distal end. The proximal ends of the pair diverge, each through an angle of 90° ; i.e., until they are 180° apart (figs. A_3 to A_5). The proximal ends now point in opposite directions, and the pair, thus attached at their distal ends, form a rod. In this condition the pair (tetrad) enters the first maturation spindle (fig. A_5).

The defective tetrad, like the others, has gone through this process. But a portion of the distal end of one of the chromosomes being gone, the chromosome cannot conjugate properly with its mate and so has a tendency to slip to one side when the tetrad reaches this stage, as figures 5 to 7, and E_2 to E_5 show. The normal conjugant (diad) finds at its distal end no corresponding portion in the defective mate with which to pair. The chromosomes were probably paired normally at the proximal ends and in all parts which are present, leaving the distal part of the larger normal member extending beyond the shortened distal end of the defective mate (fig. E_2). In the process of separation which follows conjugation the defective chromosome (diad) had its shortened distal end rotating on the side of the distal end of the longer, normal mate. When the diads had rotated apart 180° this shortened end was as a result out of line with the end of its longer mate (figs. E_2 to E_5). This is the condition in which the defective chromosome of figures 5, 6 and 7 c is seen.

2. AN UNEQUAL PAIR OF ANOTHER TYPE OCCURRING IN *ACRIDUM GRANULATUS*

While working out the chromosomes in dividing ovarian follicle cells of the female individuals of *Acridium granulatus*, I found one animal which showed among its 14 chromosomes five long members (fig. 11) instead of four (the two 6's and the two 7's). This puzzled me in my pairing of the chromosomes. The material was laid aside for the time. A few weeks later I found a male individual which showed in its first maturation divisions

the same five long chromosomes. My problem was solved at once when I saw that this extra long chromosome paired in maturation with one of the ordinary chromosomes of the complex, the No. 1 (figs. 12-13).

This unequal pair appears to be of a type different from the deficient tetrad just described, and so must be treated separately. It is of primary importance in its bearing on questions of synapsis and of maturation division. Only two animals having it were found, a male and a female. These, and most others of this species worked upon, were collected from a spot 75 feet square near Waverly, Massachusetts, and are probably related individuals.

Figures 9 and 10 show cells from normal individuals exhibiting the typical condition of the chromosomes in *Acridium granulatus*. There are two long pairs (6's and 7's), two intermediate pairs (5's and 4's), two short pairs (1's and 3's), and the sex chromosome, which in size ranks No. 2 in this genus. A small, faintly staining, fragmentary body is shown (dotted) in figure 9. It is not present in all cells, and is probably a nucleolar structure of some sort. The normal chromosomes paired for the first maturation division may be seen in figure 10. Their size relations are clearly shown there: two large pairs (6, 7), two intermediate pairs (4, 5) and two small pairs (1, 3), as well as the small 'accessory' chromosome (2).

Figures 11 to 13 are of the abnormal male and female. In figure 11 (female) the five, instead of four, long chromosomes are clearly shown. The fifth long chromosome (1) appears odd because it has no equal chromosome with which to pair. The first maturation division in the male (figs. 12-13), however, shows it pairing with the small (no. 1) chromosome. In the male cells, moreover, it shows a constriction at a region about as far from its distal end as the length of the small (no. 1) chromosome with which it is paired.

The other chromosomes are quite normal in these animals (figs. 11-13). In the female (fig. 11) there are the two long pairs (7's and 6's), the two intermediate pairs (5's and 4's), the short pair (3's), the 2's (which are the sex chromosomes and are

paired in the female), one normal No. 1 chromosome, and the abnormal (long) No. 1. In the male cells (figs. 12-13) there are two large pairs (7 and 6), two intermediate pairs (5 and 4), one small pair (3), and the sex chromosome (2) unpaired; the other small tetrad is represented by one small No. 1 chromosome paired with the abnormal long chromosome. It is evident from these two figures that this long structure is connected with one of the small (No. 1) chromosomes.

The long abnormal diad bears no constant relation to the sex chromosome in its distribution to the second spermatocyte cells, as figures 12 and 13 show. A large number of dividing cells examined showed that in the reduction division it passed as often to the pole which did not receive the sex chromosome as to that which did. I feel certain, therefore, that this chromosome bears no relation to the sex-determining chromosome.

2a. Discussion

What is the origin of the long chromosome?

The partially constricted off distal portion of this long body (figs. 12-13) is probably a No. 1 chromosome and in synapsis it was possibly paired side to side with the opposite normal No. 1, leaving the remainder of the long chromosome, the proximal end, to extend beyond it (figs. B₁ and B₂). In the Tettigidae, as I have said above, side-to-side pairing in synapsis occurs during the bouquet stage. Following this period the chromosomes of a pair separate from each other, beginning at the proximal ends. Remaining in contact at the distal ends, they continue to rotate apart until the pair appears upon the first maturation spindle as a long rod frequently constricted in the middle (figs. 10, 12, 13 and figs. A₁ to A₅). That is exactly what has occurred in the case of this abnormal tetrad, but to the end of one of the No. 1 members is attached this extra $1\frac{1}{2}$ portion, which increases the length of this member to two and one-half times its normal dimension (figs. B₁ to B₄). Unfortunately no satisfactory prophase stages are present to show the behavior of this element in synapsis, but, judging from the constriction

at one end and knowing the behavior of the unequal tetrads of other individuals (figs. 4-8), I believe that here we probably have the result of an unequal division of some preceding generation where a No. 1 tetrad failed to divide at the proper place, in the middle between the two members, but instead so divided as to give a $1\frac{1}{2}$ portion of a No. 1 pair to one pole and a half-portion to the other pole (C_1 and C_2). As a result of this, it might be imagined that there would be some generations of individuals with a sesqui-valent ($1\frac{1}{2}$ -valent) No. 1 chromosome present pairing with the normal No. 1 (figs. D_1 , D_2). The normal No. 1 might pair with the normal part, or with the fragment. In order to get a $2\frac{1}{2}$ -valent No. 1 chromosome the parts of which are largely oriented in one direction it will now be necessary to suppose that, at some reduction division of the germ cells in succeeding generations of animals, a normal No. 1 chromosome became attached during the synapsis period to this sesqui-valent No. 1 chromosome. It might do this by becoming fused with, or by failing to separate from, the fragmentary (cut off) end of the sesqui-valent member after some method as is indicated in figures D_1 to D_6 , giving as a result a $2\frac{1}{2}$ -valent chromosome (fig. D_6), such as we have in figures 10 to 13. All portions of this chromosome, except the sesqui-valent fragment in the middle, would then be oriented in one direction (see arrows in figs. C_1 , C_2 , and D_1 to D_6). My reason for thinking that at least the terminal parts, which make up the greater part of the long chromosome are oriented in the same direction, is based upon the fact that I always see the chromosome arranged on the spindle in perfectly normal position, pointing directly toward the pole to which it is about to go, and upon the fact that the mantle fiber of this chromosome always springs from the longer ($1\frac{1}{2}$) portion of the chromosome (figs. 12, 13, and B_1 to B_5). The chromosome always travels with the larger, sesqui-valent portion in advance (figs. 12, 13, B_4 and B_5).

In this animal evidently the normal No. 1 chromosome paired during synapsis with the distal No. 1 portion of the $2\frac{1}{2}$ -valent chromosome (figs. 12, 13, and B_1 to B_5). It should pair also

with the sesqui-valent portion, but I have seen no evidence that this occurs. Possibly the sesqui-valent portion has become so non-functional that it fails properly to attract the normal No. 1 chromosome in the synapsis period.

This explanation is the best I can propose, in the light I now have, to account for the origin of this long chromosome. The facts are that it is approximately two and one-half times the length of a normal No. 1 chromosome, all or most of its parts are evidently oriented in one direction, and a constriction near the distal end marks off a portion which evidently pairs in synapsis in the normal manner with the No. 1 chromosome and separates from this chromosome in the normal manner in the first maturation division (figs. 12, 13, and B₁ to B₅).

An essential fact is that we have here cases of an abnormally large chromosome, which is constant in size in individuals of both sexes. Its relative size is the same in all the dividing germ cells found in the male, and likewise in the somatic cells (follicle) of the female. I believe that it is a permanent structure, so far as these two individual animals are concerned.

A second important fact is that this abnormally large chromosome alternates with a normal chromosome and it may or may not be present in either sex. Theoretically, we may have, depending on the presence or absence of this chromosome, three sorts of male individuals and three sorts of female individuals; those containing two normal No. 1 chromosomes, those containing one normal and one abnormal No. 1 chromosome and those containing two abnormal No. 1's. I have found the first two cases in both sexes. The latter case has not yet been found in either sex. We have therefore a basis for a Mendelian ratio. The presence of one long and one short chromosome might be considered the cell condition of the heterozygote, or hybrid; the presence of two short chromosomes or of two long ones would give the homozygotes, recessive and dominant, if such relations may be imagined in so far as these chromosomes are concerned.

3. ON SYNAPSIS AND REDUCTION

In their behavior these unequal tetrads throw a good deal of light on the nature of the synapsis of chromosomes and their separation from each other in the reduction division. In regard to the beginning of this process, the individuals furnishing these abnormal chromosomes do not have much to show, as I have said above. This beginning, however, I determined from normal spermatogenesis, and have worked it out in my second paper.

I have found the pairing process in the Tettigidae to start as a parasynapsis and to end in a telosynapsis (end-to-end pairing). In the synizesis period following the last spermatogonial division, the members of each pair of chromosomes, six pairs in all, having assumed the thread condition, are seen to arrange themselves in side-by-side fashion. The number of threads is at first twelve, in addition to the accessory chromosome. The twelve threads are seen to become six double and finally six single threads. During this period the sex chromosome usually lies at one side of the nucleus. This condition of pairing continues into the later growth period, when the members of each pair move apart, the separation beginning first at the proximal ends (figs. A₁ to A₄), the chromosomes of a pair remaining attached to each other at the distal ends (figs. A₄, A₅). Each rotates through 90° and the two then form a rod (fig. A₅), which may show, by a slight constriction, the point of contact of the two members. In this condition the paired chromosomes (tetrads) enter upon the first maturation division.

In the behavior of these unequal pairs I feel perfectly certain that the same thing must have taken place, for I see no reason to think that these abnormal pairs should behave differently from the normal chromosomes during this process. Their behavior on the maturation spindle and in the anaphase following is in every way similar to that of the normal chromosomes, and we have every reason to believe that a parasynapsis ending in a telosynapsis has taken place. The unequal pairs appear on the maturation spindle in the telosynapsis condition.

Attention, first of all, should be called to the permanency of size of these abnormal pairs, especially of the abnormal members

of each pair. In the first type of inequality which I described (figs. 4-8), all dividing cells examined showed the members of the No. 4 pair unequal and constantly of the two respective sizes. The normal No. 4 chromosome was always of the size normal for No. 4 chromosomes and its deficient (No. 4) mate was likewise of a constant size but uniformly one-sixth less than the normal No. 4 in all cells, both somatic and germinal. The constancy of this relative inequality was very clearly shown, especially in those figures of the first spermatocyte division which appeared in lateral view (figs. 5-8). A similar uniformity of sizes I found in the unequal pair of the second type (figs. 12, 13) in both male and female animals. Abundant evidence of the relative sizes was given, especially in the tetrads of the first spermatocyte divisions. Every cell showed the No. 1 portion of the abnormal tetrad to be of the normal No. 1 size (figs. 10-13) and the longer $2\frac{1}{2}$ -valent portion to be likewise of a constant size. It was not only of uniform size in all the cells of the male individual, both somatic and germ cells, but in another individual, a female, it was also found to be of the same length (fig. 11).

These facts indicate that in the latter case this abnormally long chromosome must have been handed on as such, not only through many generations of cell division, but also through many generations of individual animals. In the former case the deficient No. 4 chromosome—which was of practically the same size, not only in a large number of spermatocyte cells, the germ cells, but also in the body cells—indicates again that such an abnormal chromosome maintains its identity from generation to generation, from fertilized egg to fertilized egg.

The permanency of these abnormal chromosomes has an important bearing upon one problem of synapsis; upon the question as to whether or not a complete side-to-side fusion of homologous chromosomes takes place during this period. I have attempted to illustrate my ideas by diagrams (figs. F_1 to F_5 , G_1 to G_6 , and H_1 to H_6). If we suppose that a fusion of the chromosomes takes place, we should expect to find in the case of the unequal No. 4 pair something like figures F_1 to F_3 occurring. The shortened No. 4, pairing with that portion of the normal

No. 4 to which it corresponds, or with which it is homologous, and leaving the distal end of the normal No. 4 projecting beyond (fig. F_1), would, on fusion with it, form a single cylindrical body having one end, the distal, smaller in diameter than the remainder of the cylinder. If a splitting occur at the end of synapsis in a plane formed regardless of the old plane of fusion, having merely for its object the splitting of this single fused body into symmetrical halves, there would result two daughter chromosomes of equal size but with smaller diameter at the distal end than throughout the remainder of the chromosome (figs. F_3 to F_5). There would then be no such uniform inequality of the No. 4 chromosomes preserved in the first spermatocyte divisions of this animal as we have found. Instead of having a normal sized No. 4 and a (say) five-sixth-sized No. 4 in every cell after the first maturation division, there would be two No. 4 chromosomes of equal length but shorter than the normal (fig. F_5). On the contrary every first spermatocyte metaphase and anaphase (figs. 5-7) shows one normal sized No. 4 chromosome of the same fixed relative size and likewise one deficient No. 4 chromosome of a definitely fixed relative size.

If we turn to the abnormally long tetrad (figs. 12, 13), we have even more definite proof that fusion longitudinally and splitting along a new plane in all probability does not occur. The normal No. 1 chromosome evidently has paired with the distal portion of the long No. 1 chromosome in a manner similar to the diagram shown in figures B_1 and B_2 . If in such pairing it fuse completely with its longer mate, it would form a cylinder again, with one end of larger diameter, the opposite of smaller diameter (figs. G_1 to G_4). If, on the splitting of the fused thread, the new split be formed, not along the old plane of fusion, but upon any plane giving two equal daughter threads, we would get two long chromosomes of the same length with diameters large at the distal end instead of a long chromosome and short chromosome, of the same relative lengths and equal diameter, with which we started. Again, if the new split appeared in the fused chromosome in a plane at right angles to that of fusion (figs. H_1 to H_6), we should

get a similar result, which is also contrary to the facts in the case. The chromosomes, when they come out of the pairing process, are evidently of the same size they were on entering the process. The abnormal sized $2\frac{1}{2}$ -valent No. 1 chromosome is of the same size in all the germ cells after the process as in the germ cells of the same individual before the process, and even in the body cells of another individual (fig. 11). The same may be said of its normal mate. No tags or projections were seen in any case to indicate that a new longitudinal plane of division had occurred.

In both types of unequal tetrads we have very strong evidence that homologous chromosomes, on entering the side-to-side pairing process of synapsis, remain as distinct individuals, retain their identity throughout the period and come out of it with at least the same size they had on entering it. Each pairing chromosome maintains its distinct individuality during this period.

This is opposed to the ideas of Jannsens ('09) and Morgan ('11) as expressed in the theory of the 'chiasma type.' In their theory they assume that homologous chromosomes in parasynapsis twist about each other and fuse. On splitting, a plane passes down the fused body, regardless of the previous spiral fusion-plane, resulting in two daughter chromosomes which may not be identical with the two chromosomes which entered the process. Each new one may contain parts of both original chromosomes. If such had been the case, the separation or formation of a short and a long chromosome out of the fused chromosome (B_2 to B_5), with such regularity of size, etc., as we have shown, could not have occurred. Or, supposing that spiral twisting and fusion had occurred and that splitting again was limited to the larger end of the fused chromosome (figs. G_2 to G_4), we should expect that the shorter member resulting (figs. B_2 to B_5) would at least show fragments of the side of the long chromosome attached to its proximal point in those cases where the splitting plane did not coincide with the old plane of fusion between the short and long chromosomes. No such attached fragments were found. All short or normal No. 1 chromosomes in the metaphase and anaphase of the first spermatocyte divisions were of uniform length

and showed that they retained the same size and were as free from terminally attached fragments as they were on entering the pairing process.

In regard to the question of pre- and post-reduction, I have evidence here that the first maturation division is the reduction division so far as regards three of the seven pairs of chromosomes of the Tettigidae subfamily of grasshoppers. These pairs are the 1's, the 4's and the sex chromosomes. The abnormally long No. 1 chromosome is seen in Tettigidea separating from its normal mate (figs. 12, 13), the deficient No. 4 is seen separating from its normal mate (figs. 5-8), and the unpaired sex chromosome (paired in the female, figs. 1, 11) is seen passing over whole to one of the poles in the male cells in similar manner, just as if it had had a mate from which to part at this division, where such parting is being carried out by the members of the ordinary chromosome pairs (figs. 2, 3, 5, 6, 8, 10, 12, 13). The other chromosome pairs (the 2's, 3's, 6's, and 7's) behave similarly to the 1's and 4's in every respect during the synapsis, prophase, metaphase, and anaphase periods of the first spermatocyte divisions. This leads one to think that probably the first spermatocyte division is the reduction division for all pairs of chromosomes, in the Tettigidae subfamily of grasshoppers at least.

4. THE DEFICIENT NO. 4 CHROMOSOME AND THE LOSS OF UNIT CHARACTERS

The most important of these unequal tetrads are those in which one member of the pair is of less than the normal size (figs. 4-8). The importance lies in this, that such a deficiency may be the result of the dropping of a part of a chromosome and in this way may furnish the basis in the germ cell for the loss of unit factors in heredity.

In animal and plant breeding there are many unit characters which might be considered to have resulted from the loss of something from the germ plasm, whether this something be an enzyme of some sort or a substance upon which an enzyme might act.

It has been shown by Castle ('11) and others that in mice, rabbits, and guinea-pigs the ordinary gray color of the wild rodent is not a simple character, but on the contrary, is very complex. It depends—for example, in mice—upon the simultaneous presence of at least seven or eight color factors. The complete dropping of any one of these from the germ plasm modifies the color of the animal.

The factor most commonly dropped is that for the production of color, the animal—whether potentially gray, black, or brown, etc.—being an albino, actually without color. The next factor very commonly dropped is that for barring of the fur. This does not show unless black or brown pigment is present in the hair. The barring of the hair is due to the fact that some factor prevents the development of black and brown pigment granules in a portion of the individual hair immediately below the tip, leaving a black tip and a dark base with a light yellow band between them. When this barring factor is dropped, we get a black or a brown animal instead of a gray. The next most commonly dropped factor is that for the production of black, giving cinnamon or brown animals. By dropping the factor for self color, spotting results, spotting of two sorts, white upon a colored coat, or yellow upon a black, brown, or gray coat,—giving in the extreme cases white with black or brown eyes and yellows with black or brown eyes. By dropping the dark-eyed character we obtain pink-eyed animals with a scarcity of pigment in the fur. By dropping the intensity-of-pigmentation factor we get diluteness of pigment, giving dilute-pigmented gray, black ('blue'), brown ('cream'), or yellow animals. When all of these factors are present we have the wild gray type. By the dropping of any one of them entirely the color may be modified accordingly.

It is evident that these animals lack something. The dropping of the black pigment, for example, gives animals which are cinnamon or brown. No black can be produced in the race until this factor is again added to the mixture. It is entirely absent. In this case black cannot be considered a latent character which has been restricted in some way from attaining its development, as is the case in albinos of gray or black animals;

these on being crossed with brown, or any animal that contains the color factor, will give gray or black, etc., showing the gray or black characters to have been present in substance though not developed. If the black be absent, nothing can restore it except the addition of black again. There is evidently a complete absence of the black pigment material from the fur of the animal.

In the varieties of domestic plants many similar cases may be given. But before mentioning the color varieties I wish to call attention to the cross between the 'cupid' and the 'bush' variety of sweet pea as one of the best examples. The cupid is a dwarf plant whose internodes are very short, making the stem correspondingly short, about 9 to 10 inches. It has the prostrate habit, that of lying on the ground, due to the diverging habit of the branches. The 'bush' variety produces branches which do not diverge but grow upright, making a tall bush growing 42 to 48 inches in height. These varieties, on being crossed, produce in the F_1 generation plants which show a reversion to the habit and size of the wild sweet pea of Sicily (Punnett '11). They have the long internodes and the long stem of the bush variety combined with the prone, prostrate habit of the cupid variety. By inbreeding the F_1 individuals the F_2 show the tall 'bush' variety, the tall procumbent, the short procumbent or original 'cupid,' and the short 'cupid' bush-like variety. The factors concerned are the long internodes versus the short, and the procumbent versus the erect habit. The two varieties evidently owe their origin to the dropping of one or the other of these factors from the germ plasm of the wild type. The bringing of them together in the hybrid supplies the lack in both races (the 'cupid' and the 'bush' varieties), the result being the wild type.

In flower color of sweet peas we have a similar case. Most white sweet peas breed true to white, but there are two varieties of white which on being crossed produce a purple colored variety, like the wild Sicilian species. On being inbred, the F_2 generation shows nine of the colored variety and seven of the white. Of the whites some breed true, some give a 3.1 ratio, i.e., three whites

to one colored, while some give all colored again. This shows that there are two factors concerned. The presence of both is needed to produce the colored flowers, the absence of either one giving white. It is the F_2 result of an ordinary dihybrid Mendelian ratio, in which there are nine cases where both factors are present and colored results, three cases where only one factor is present and white results, three cases where the other factor is present and white results, and one case where neither factor is present and a white which breeds true results.

The colored individuals in the F_2 generation are found to belong to six classes depending upon the presence or absence of a purple factor, a light-wing factor giving a bi-colored flower, and a factor for intensity of pigmentation, in the absence of which a dilutely colored flower results. The wild type is intensely colored, and bi-colored, i.e., purple with blue wings. By dropping the purple a red bi-colored or uniformly colored flower is obtained in both the dilute and intense series, by dropping the bi-color factor uniformly purple or red are obtained, by dropping the intensity factor both varieties of the red are obtained in dilute form, and, finally, by dropping either the color-producing base or the color developer we get any of these color varieties in the albino form. The wild purple Sicilian species contains all these factors. By the dropping of these factors one by one and by inbreeding, all the color varieties of our domestic sweet peas have been obtained.

Now, it seems to me that it would be quite possible to account for phenomena of this kind in plants and animals as the result of an unequal division of the chromosomes in the reduction division, similar to what I have found evidence of in *Tettigidea parvipennis*. If but one member of the pair of chromosomes showed the deficiency it might not give a result in the organism. When both chromosomes of the pair show such a deficiency, a condition which would result only when inbreeding occurs, then such a deficiency might be shown in the somaplasm by some such defect as albinism, blackness, dwarfishness, etc. It is true that such a chromosome would evidently be an abnormality of a deficient kind, but are not all such traits as albinism in plants

and animals, melanism in rodents, retarded mental development, feeble-mindedness in man, dwarfisms, etc., abnormal deviations from the type of the species which may be classed as deficient characters?

The amount which may be dropped from one chromosome, such as I have shown in *Tettigidea*, may be considered too much to allow of the existence of the organism in the homozygous condition, i.e., where both members of the pair lack it. It is conceivable that a limit in the amount that may be absent might occur and that, going beyond that limit, the homozygous condition might be lethal, as in yellow mice. Here the homozygote always dies, or may never be formed, as has been shown by the results obtained from breeding yellows together. The litters are three-quarters the normal size and the young are found to be two-thirds yellow and one-third gray or other colors, never breeding true to yellow. This indicates that the yellow parents were heterozygotes and that the one-fourth pure yellows, which we would expect in F_2 , have never been formed. Baur ('07) found, on crossing two varieties of snapdragon—the green leaved with the golden leaved—that the offspring were 50 per cent green and 50 per cent golden leaved. The greens bred true but the golden variety produced 25 per cent greens, 50 per cent golden and 25 per cent that were almost white. The latter died at the end of germination, when the food in the seed was used up, since they possessed no chlorophyll. The golden variety was variegated with green patches (chlorophyll-containing cells) and so could manufacture its own starchy food.

The yellow condition in mice and in the snapdragon may be due to a greatly deficient chromosome, so greatly deficient that a zygote having two such deficient chromosomes might have too great a deficiency to be able to develop, or, after having developed, may lack some substance, such as the chlorophyll in the snapdragon, necessary for carrying on one of the vital life processes. In addition, this lack might be so great that it would over-ride the normal chromosome in the heterozygous zygote, giving a yellow mouse instead of a gray, black, or brown, as the case may be; or in the snapdragon a golden variety instead of a green

variety. That something of this nature has occurred, we are inclined to believe from the fact that the golden variety is simply a plant which lacks chlorophyll in small variegated patches over its surface, whose color is therefore due to this condition. In the cases here given possibly there is a law at work correlating the amount of material that may be lacking from a chromosome with the recessiveness or dominance of the trait resulting in the organism. A defective chromosome may continue to give a trait which is recessive to the normal condition until the defectiveness of the chromosome reaches that point where its deficiency becomes so great that the homozygous zygote cannot develop. At that point the defect becomes dominant to the normal condition and individuals can exist only in the heterozygous or normal condition.

It is strange that yellow should be dominant in mice while in most other species of domestic animals it is recessive. This may be due to the position of the yellow determinant along the chromosome. In most species it may be thought of as lying near the end of the chromosome and accordingly could be dropped very easily, causing little disturbance and giving a recessive trait. In the mouse it may be conceived of as lying farther from the end of the chromosome. On dropping enough of the chromosome to cause yellow, a greater disturbance would be created and the defective trait resulting would accordingly be dominant to the normal trait. I make this as a suggestion merely.

The chances for such abnormal divisions are limited by the number of pairs of chromosomes in the species and by the varying amounts which may be dropped from each chromosome in each case. In guinea-pigs the number of pairs is twenty-eight (Stevens '11). The chances for abnormal divisions in guinea-pigs are therefore large. Where the number of chromosomes is small the chances are smaller. The amount that may be cut off from each individual chromosome might vary enough to give several varieties due to the variation in this respect in one pair of chromosomes. The same might be said of each of the twenty-eight pairs in guinea-pigs. Any of these varying conditions in a single pair of chromosomes might combine with

all possible combinations of similar variations in the twenty-seven other pairs. Such might be the basis of all of our variations in guinea-pig inheritance.

It is noticeable in any animal or plant which becomes domesticated that very soon there appear whites, blacks, browns, spotted, yellows and others of the color varieties common to domesticated species. The same may be said of other characters of the species, size for instance. On domestication inbreeding occurs. This gives rise to homozygous strains, which may be isolated. In the wild state inbreeding is not so prevalent. Promiscuous mixing occurs. A summing up of the characters results and all normals, which are usually dominant, are present and show. The weaker recessive characters, if present, are covered up. They exist in the single or heterozygous condition and so do not show. If they exist in the homozygous condition they may show, as in albinos, but the organisms may be killed off by natural selection. Under domestication man preserves these recessives.

The loss of parts of chromosomes may explain very easily the appearance of such phenomena in the domestication of species. The fact that there appear always the same or nearly the same color varieties in each species may be due to this, that their chromosomes are more or less similarly organized, that there are approximately the same number and in many respects the same individual chromosomes to be dealt with in each species, and finally, that these chromosome pairs are subject to the same vicissitudes of fortune in division at the maturation period in each species. True, there are minor variations in chromosomes as we pass from species to species. There are also minor variations in color inheritance as we pass from species to species. But these variations are small when we think of the similarities. All species of rodents show grays, all show albinos, all show blacks, all show some form of brown, and yellows. The method of inheritance of the yellows, for instance, might vary from species to species, but they are yellow, nevertheless.

The same spontaneous variations, such as albinism, probably occur not once but over and over again in the same species in various parts of the world entirely independently of each other.

If the basis of albinism lie in an abnormal reduction division of a certain pair of chromosomes, we should expect it to do just that sort of thing. So long as the same number of pairs of chromosomes occurs in a species, so long will the same variations continue to occur. In this way we shall continue to have white animals produced anew by 'mutation'—blacks, yellows, spotted, and all the varieties not only of color but in respect to other properties of the body as well. In the same way we might always expect to have produced a certain percentage of defective human beings, such as the classes of feeble-minded, imbeciles, epileptics, etc., each of which seems to be due, in many cases at least, to something lacking in the germ plasm (Davenport '11).

Germinal variations of this kind, it seems to me, might be at the basis of De Vries' 'eversporting varieties,' which gave such abnormalities as striped flowers, five-leaved clovers and monstrosities of various sorts, such as pistillody, twisted and flattened stems, etc. Again, it seems possible that germinal variations of this sort might lie at the basis of those of De Vries' mutations which he distinguished as retrogressive in character: i.e., which were characterized by the dropping out of some character from the parent species. They might lie at the basis of some mutants which he considered progressive, but which showed some retrogressive traits, such as the brittleness of the stem in *Oenothera rubrinervis*. My reasons for supposing this are as follows: De Vries found his 'eversporting varieties' producing their abnormal individuals continually. He found his parent species, *Oenothera lamarckiana*, continually throwing off the same mutants in certain proportions. "No single parent plant proved ever to be wholly destitute of mutability." In his parent species, *lamarckiana*, he has probably a constant fundamental number of chromosomes to deal with. He has a reduction division taking place every time a germ cell is formed. He has the same possibility of abnormal, unequal, divisions of tetrads at the time of this division, giving a deficient homologous chromosome. He has self fertilization (inbreeding), which would tend to bring such defective chromosomes together. He has frequent cases of sterility in the inbred offspring of his mutants,

as one would expect in instances where a vital factor had been dropped. He has mutations which, with one or possibly two exceptions, are of a retrogressive nature; i.e., lacking something necessary which was present in the parent species. The gigas variety was due evidently to a doubling of the number of chromosomes (Gates '09). It seemed to lack nothing, but the other mutants seemed all to lack traits, some more useful, some less useful, which were present in the parent species. These phenomena, it seems to me, point to something for their basis like the abnormal variations in reduction divisions, such as I have described in *Tettigidea parvipennis*.

It is interesting to compare the number of mutations De Vries obtained from *lamarekiana* with the number of chromosomes. His number of chromosomes was 14, seven pairs. He obtained seven mutations from his plants. One of these, gigas, was evidently due to a doubling of the number of chromosomes. It, however, was not a defective mutant, and so may be left out of account here. The other mutants seemed to have something lacking, and there were six of them. Of these scintillans seems to have been heterozygous, producing on inbreeding, *lamarekiana* and scintillans. It also produced, in a small percentage, some of the mutants most frequently produced by *lamarekiana*. Possibly scintillans had one over-deficient chromosome, such as I have postulated for yellow mice or the golden snapdragon. Many sterile pollen grains were found. Possibly the cause of sterility lies here. On this hypothesis, the fact that scintillans can produce *oblonga*, *lata*, and *nannella* is not surprising. The other chromosome pairs are just as liable to accidents in germ cells of scintillans as of *lamarekiana*. *Lamarekianas* may be produced by scintillans; why may not the mutants of *lamarekiana* be produced also? Each of the other five mutations might be based respectively upon deficiencies in one of the remaining five pairs of chromosomes. Since these remaining mutants breed true in each case, it would be supposable that, in order to show, they must be in the homozygous condition. Thus we may possibly account for the five remaining mutants. This hypothesis, it seems to me, is worthy of consideration here. I

see many reasons to suppose that De Vries was dealing, in part of his mutants at least, with something similar to what I have described in this paper as deficient homologous chromosomes.

Deficient chromosomes, such as I have found paired with their normal sized mates in *Tettigidea parvipennis*, it seems to me, furnish a sufficient explanation for the loss of unit factors from the germ plasm. Looked at in the light we now have of the behavior of unit characters which belong to this 'loss' group, the hypothesis appears very probable. It seems to me that Professor Morgan and his students, who are working upon *Drosophila*, should also take into consideration the possibility of such deficient chromosomes. It is a good working hypothesis, and I am going ahead with breeding experiments upon this species, *Tettigidea parvipennis*, in the hope of getting some results. As to what connections there may be between these deficient chromosomes and the greater problem—the origin of new characters—it is difficult to imagine. This matter had better be left until we have more knowledge of the behavior of these chromosomes.

The observational work upon which this paper is based was done at Harvard University under the direction of Prof. E. L. Mark. The writing was completed at Kansas University. I wish to express here my gratitude to Dr. Mark for the help and criticism he has so kindly given to me from time to time.

ADDENDUM

After this paper was worked out, a treatise by Miss Carothers ('13) appeared, describing unequal tetrads in three species of the 23-chromosome grasshoppers, *Brachystola magna*, *Arphia simplex*, and *Dissosteira carolina*. I wish to consider her paper briefly here.

In the twenty specimens of the three species examined she finds the members of one of the three pairs of small chromosomes always unequal in size. The unequal pair occurs in spermatogonial, in first spermatocyte and (separated) in second spermatocyte cells. The members of the unequal pair agree with those I have found, in that they become separated from each other during the first and not during the second maturation division. This is evidence again that the first maturation division is the reducing division. In their passage to the second spermatocytes these unequal members (diads) agree with those of my material in that they are distributed to these cells irrespective of the presence or absence of the sex chromosome. In this respect our unequal pairs differ from those of *Gryllotalpa*, described by Payne ('12), where the longer chromosome was always accompanied by the sex chromosome in the anaphase of the reduction division. Payne may have been dealing with a group of sex chromosomes similar to what he has already worked out in another order of insects ('09).

Miss Carothers' material differs from mine, however, in that she finds the unequal pair present in every animal. I cannot agree with her in this respect, in *Acridium* or *Tettigidea*, as my drawings have shown. Possibly further search will show that the unequal pair is not always present in the species upon which she has worked. If this should be the case, the hypothesis of selective fertilization which she advances would be unnecessary. So far as *Tettigidea* and *Acridium* are concerned, it is not necessary to postulate selective fertilization.

In thinking that the great number of combinations of chromosomes possible is sufficient to account for all variations, I fear Miss Carothers may be mistaken. Gates ('09) has shown that in

Pisum the number of pairs of chromosomes (seven) is not large enough to account for the number of pairs of allelomorphic characters which behave independently of each other in breeding experiments, if we assume that the basis of each member of one allelomorphic pair must be permanently located in one member of a single pair of chromosomes:

In Pisum eleven or more pairs of allelomorphs have been observed and the reduced number of chromosomes is only seven; which shows that in this case at least, several characters must reside in one chromosome. The characters must then be confined to separate particles or corpuscles of the chromosomes, and an interchange of homologous particles according to chance during maturation would give the Mendelian combinations.

I am not quite willing to believe that the basis of an allelomorph may slip from one chromosome to another. Yet it is very evident, so far as I can see, that the number of chromosome pairs behaving independently of each other is too small to allow them to be the basis for the number of allelomorphic pairs of characters behaving likewise independently of each other. Possibly some of these extra pairs of allelomorphs may be accounted for by the deficient chromosome hypothesis which I have advanced, or possibly by the 'chiasma' theory of Morgan, though I have evidence against the latter in these unequal chromosomes and in the V-shaped chromosomes of *Chorthippus* and *Jamaicana* (Robertson '15).

Additional instances of unequal chromosomes, so far as I have been able to find in the literature, have been reported by Baumgartner (Science '11), Hartman ('13), and, I have been informed, by Voinov ('12). The last mentioned paper is published in a European journal to which I have been unable to get access. Baumgartner reported an unequal pair in *Gryllotalpa*, but he gave no drawings and no description. Since that time, Payne ('12) has shown that the unequal pair in *Gryllotalpa* is related to the sex chromosome, the larger member of the pair going with it in the reduction division.

A short time ago Mr. F. A. Hartman called my attention to the fact that he had already described unequal divisions of some

of the small chromosome tetrads in the first spermatocyte cells of *Schistocerca* in his paper (March '13) on "Variations in the size of chromosomes." His paper deals chiefly with variations in size of chromosomes due to, what he believes to be, their unequal growth in the cell. Thinking that the whole paper was devoted to 'variation due to unequal growth,' I overlooked the latter part in which he illustrates and describes briefly a few cases of what he considers unequal division in the first spermatocyte. One of these cases (his fig. 86) may possibly be due to faulty conditions of sectioning. The other cases (his figs. 83, 85 and 87 to 91) are probably variations similar to those Carothers has since described in *Brachystola*, *Arphia*, etc. That "size variations may be due to unequal growth" I am inclined to doubt, but Hartman is to be given credit for recognizing the importance of variation in chromosome size in its relation to 'variation' in 'animals,' since his work was done while teaching in a high school away from any university contact and especially since he was entirely ignorant of the Mendelian laws and their relation to variation. Had he known of these and the related work, he would likely have come to the same conclusions that I have.

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EXPLANATION OF PLATES

The drawings of plates 1 and 2 were outlined with an Abbe camera lucida at a magnification of 3900 diameters obtained with a Leitz 2 mm. oil-immersion objective and a Zeiss $\times 18$ compensating ocular, with draw-tube set at 150 mm. and drawing made at the level of the base of the microscope. In the process of reproduction, they have been reduced one-third, and therefore appear at a magnification of 2600 diameters. The numerals affixed to the chromosomes indicate their relative sizes, the smallest being numbered '1.'

PLATE 1

EXPLANATION OF FIGURES

1 to 8 *Tettigidea parvipennis*

- 1 Chromosomes of an oogonial cell; female.
- 2 First spermatocyte; tetrad No. 4 is normal; No. 5 is the sex-chromosome; male.
- 3 Chromosomes of a first spermatocyte of a third animal (male) showing a normal No. 4 tetrad; No. 5 and No. 2 seen somewhat foreshortened.
- 4 to 8 From a fourth animal, male.
- 4 Chromosomes of a spermatogonium; all chromosomes are split and in metaphase; one of the chromosomes of pair No. 4 is deficient.
- 5 First spermatocyte; the deficient No. 4 (4-) separating from its mate (4).
- 6 First spermatocyte; deficient No. 4 (4-) in abnormal, oblique contact with its mate (4).
- 7 Deficient No. 4 tetrads taken from three other first spermatocyte dividing cells in the same animal, showing uniform size but variation in manner of contact of the conjugating chromosomes.

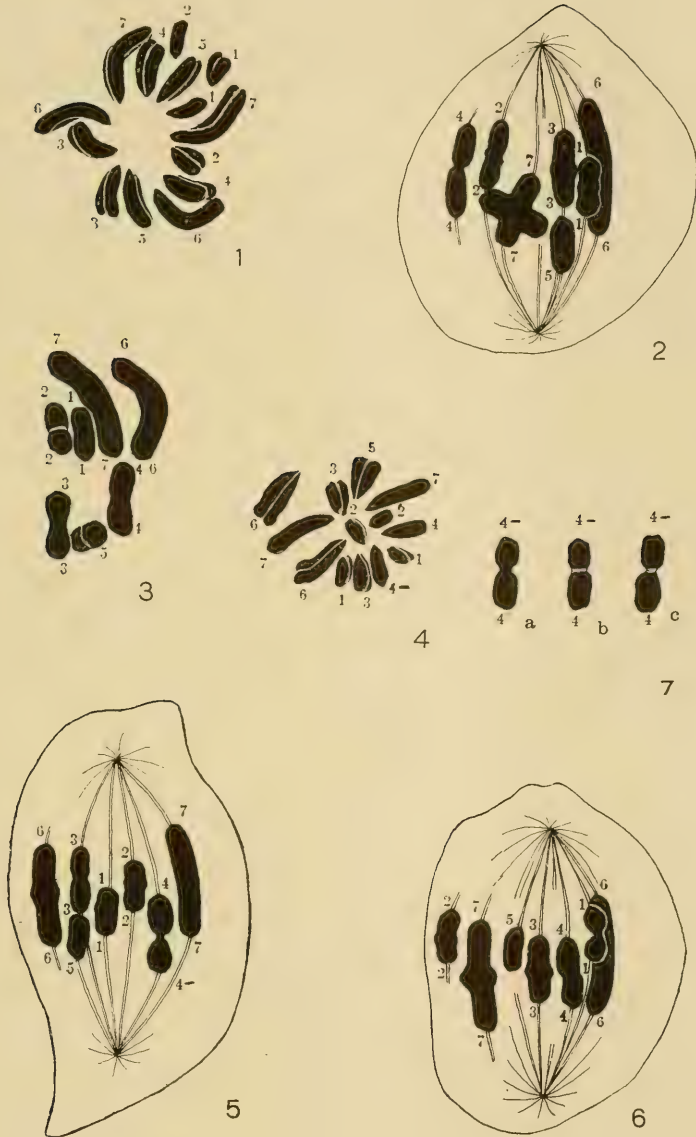
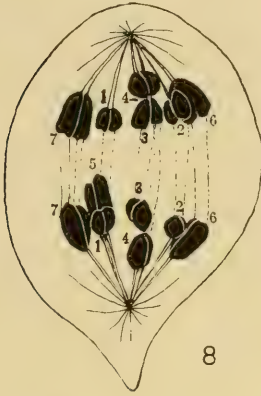


PLATE 2

EXPLANATION OF FIGURES

- 8 Deficient chromosome (4—) in the anaphase of the first spermatocyte.
- 9 to 13 *Aceridium granulatum*.
- 9, 10 From two normal males.
- 9 Spermatogonium; No. 1 chromosomes normal.
- 10 First spermatocyte, No. 1 tetrad normal.
- 11 Follicle cell of female. One abnormally long ($2\frac{1}{2}$ -valent) No. 1 chromosome.
- 12, 13 From abnormal male.
- 12 First spermatocyte, the $2\frac{1}{2}$ -valent, abnormal, No. 1 (1) separating from its normal mate (1); going with the sex chromosome.
- 13 First spermatocyte, the $2\frac{1}{2}$ -valent No. 1 chromosome not going with the sex chromosome.



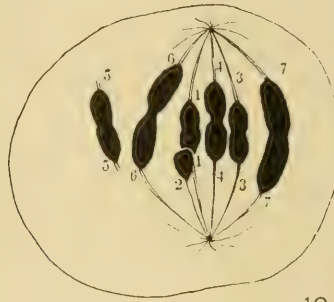
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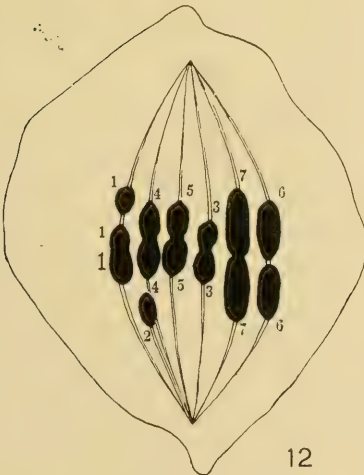
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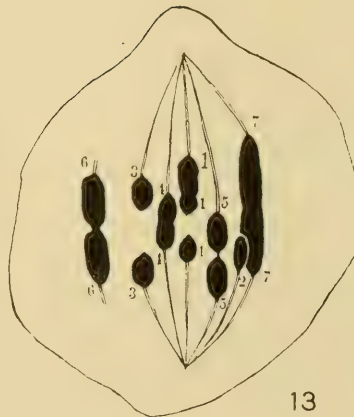
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PLATE 3

EXPLANATION OF FIGURES

A₁ to H₆, schematic

A₁ to A₆ Illustrating pairing in synapsis and separation at first maturation division of homologous chromosomes in Tettigidae. Arrows indicate direction of orientation of the chromosomes; lines and dots indicate respectively maternal and paternal origin of the pairing chromosomes.

B₁ to B₅ Illustrating manner in which the 2½-valent abnormal No. 1 chromosome pairs with and separates from its normal mate; arrows indicate orientation of parts.

C₁ to D₆ A supposed method of origin for the 2½-valent No. 1 chromosome.

C₁ C₂ Accidental unequal division in the reduction mitosis, giving a sesqui-valent No. 1 chromosome; arrows indicate orientation of parts.

D₁ Pairing in synapsis of the sesqui-valent No. 1 chromosome of C₂ with a normal No. 1.

D₂ D₃ Separation in late synapsis.

D₃ to D₅ Imagined revolution of the normal No. 1 about the fragmentary end of the sesqui-valent No. 1, giving a 2½-valent No. 1 chromosome having the two end portions oriented in the same direction.

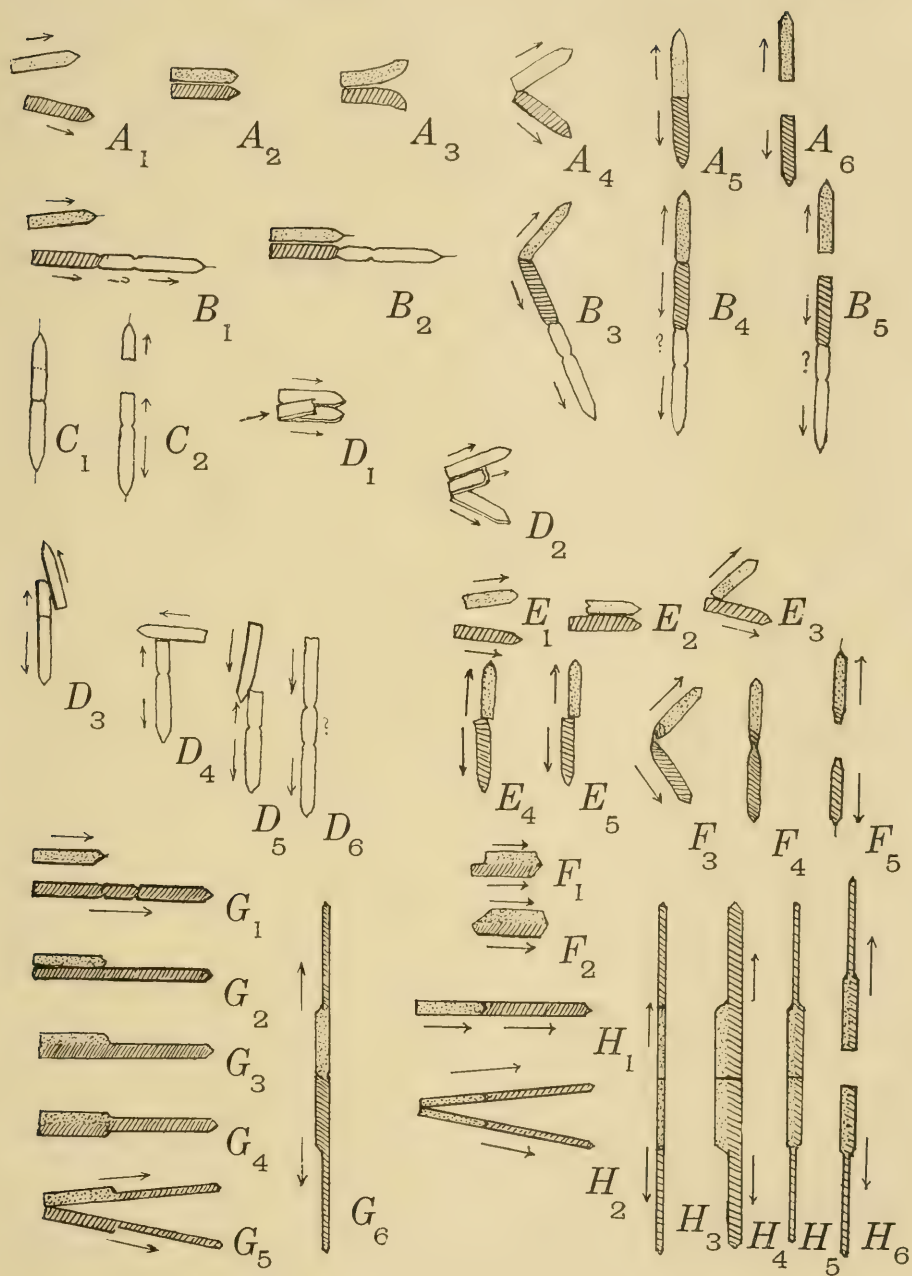
D₆ Carried over whole in a reduction division giving a 2½-valent No. 1 chromosome. The distal No. 1 portion corresponds to that part of the long chromosome in B₁ to B₂, which pairs with the normal No. 1.

E₁ to E₅ Evident method of pairing of the deficient No. 4 of figures 4 to 8. Normal No. 4 projects beyond its deficient mate (fig. E₂) at its distal end. In separation the deficient mate evidently has rotated on the side of this projecting end, and has come into position on the metaphase spindle out of line with its normal mate; compare figures 5, 6, 7 b, and 7 c.

F₁ to F₅ Showing result expected if in parasynapsis on the pairing of the deficient No. 4 with its normal No. 4 mate there occurred a complete fusion followed by a splitting of the fused body into two symmetrical parts. The chromosomes would be alike in size in the anaphase of reduction (contrary to fact).

G₁ to G₆ Showing expected result if the same as above took place in the pairing of the 2½-valent No. 1 with its normal No. 1 mate. There would be two long chromosomes of equal length having clubshaped distal and slender proximal ends, the short No. 1 getting a portion of the long No. 1 (contrary to fact).

H₁ to H₆ Showing the result if the 2½-valent and 1-valent chromosomes paired and separated in a plane at right angles to the above split (figs. G₁ to G₆), the plane of splitting not agreeing with the plane of fusion of the short and long chromosome (fig. G₂). Two equal chromosomes would result each composed similarly of like parts of both (contrary to fact).



THE EMBRYOLOGY OF BDELLODRILUS PHILADELPHICUS

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TWENTY-SIX TEXT FIGURES AND EIGHT PLATES

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INTRODUCTION

The Discodrilidae are, in many ways, extremely favorable for the study of annelid development. The material can be readily obtained at almost any season of the year. The development of any one egg can be followed from the time of fertilization to its complete development. The smallness of the eggs and the chitinous-like cocoon are the most objectionable features to contend with. Notwithstanding these facts, this group of annelids has been almost completely neglected by investigators in the study of cell lineage.

Moore, in his paper on "The anatomy of an American Discodrilid (*Bdellodrilus illuminatus*)," refers incidently to his 'embryological studies' in the course of his investigations, but has published no account of the Discodrilid development.

Salensky, in his paper on "The development of *Branchiodella* an European Discodrilid, parasitic on *Astacus fluviatilis*," gives an account of the cleavage, axial relation, origin of the germ layers and the formation of the adult structures. But the points of chief importance are so inadequately described and imperfectly worked out, that his results have no special significance in the study of Discodrilid development.

The development of *Bdellodrilus philadelphicus*, one of the Discodrilidae, has, so far as I can learn, never been worked out. It is in many respects a very important form, not only from the standpoint of development, but from its adult anatomical structure as well.

The more important points in the following paper may be briefly summarized as follows:

1. The cleavage is unequal and regular, but may be variable in some eggs. A very small cleavage cavity is present. The gastrula is formed by the epibolic process. The blastopore occupies a very small area on the ventral surface. Its point of closure corresponds to the median ventral side of the adult worm.

2. The early cleavage planes are definitely related to the future organs of the adult worm; i.e., every cleavage foreshadows

the position of some definite future formation. The large macromere D after the formation of d^3 divides very unequally, the smaller cell becomes the entomere D and the larger cell becomes the mesomere d^4 (M). The entire mesoblast is derived from the large cell M after its equal cleavage. The primary mesoblasts M, M, or meso-teloblasts, are completely grown over by the derivatives of X and the cleavage cells of the third generation of ectomeres. The descendants of the primary mesoblasts are differentiated into two distinct groups of cells. The first group becomes the dorsal mesoderm of the adult worm. The second the mesodermal germ bands, becomes the ventral and lateral mesoderm. The cells of the first group remain undifferentiated until late development. The latter becomes differentiated into muscle tissue much earlier than the former.

3. When completely formed, the germ bands consist of three distinct strata of cells: (a) An outer stratum, ectoblast from one to two cells thick, which is produced by the three generations of ectomeres. This layer persists as the definitive hypodermis and secretes the cuticle; (b) A middle stratum, which gives rise to the nervous system and the nephridia; (c) An inner stratum, mesoblast which gives rise to all of the mesodermal elements, blood vessels, septa, reproductive organs, etc.

4. The middle stratum is composed of eight distinct longitudinal rows of cells, which at first lie at the surface and form part of the general ectoderm (ectoblast), but afterwards become completely covered over by the ectoderm. There are four rows in each germ band, terminating at the posterior end in a large cell or teloblast. The inner or ventral neural row on each side gives rise to the corresponding half of the nervous system. The three remaining rows of cells (nephridial) on either side, give rise to the nephridia. The outer nephro-teloblast often proliferates but few cells.

5. The brain or cephalic ganglia take their origin from the extreme anterior ends of the neural rows and are distinctly independent of the ectoderm.

6. The cleavage of the entomeres A, B, C, and D is continued to the end without delay. The entire digestive tract,

with the exception of the very short stomodaeum and proctodaeum is derived from the entomeres. The proctodaeum is on the dorsal side of the tenth segment. The stomodaeum is formed at the apical pole. The embryo is completely turned on itself, i.e., the extreme anterior and posterior ends are in immediate contact. The outer or curved surface, becomes the ventral side of the future adult worm.

NATURAL HISTORY

The Discodrilids occupy rather a unique position in the annelid group. They resemble the Hirudinea in their parasitism, in their general shape, in the presence of an anterior and posterior sucker and in the existence of chitinous jaws. The last character is not found in any other oligochaet, but occurs in a large number of leeches. These facts, perhaps not important in themselves, are indications of a very close relationship between the Discodrilids and the Hirudinea, a group which they approach, not merely in such habits as the formation of the cocoon in which the eggs are enclosed, but in many other points of internal and external structures. The fundamental differences between the two groups are not numerous and are not of such importance as has been assigned them by different writers. The Discodrilids are classified as a distinct family of the Oligochaeta.

Bdellodrilus philadelphicus occurs very abundantly on *Cambarus virilis*, especially in the early spring and summer months. A few may persist throughout the entire winter in their natural habitat.

For convenience, the animal may be divided into three distinct regions; the head (pharynx), the body proper, and the posterior sucker. The head is much broader than the anterior body segments. The head is composed of four distinct annuli, which perhaps represent distinct segments. The first or peristomal annulus is divided into very mobile dorsal and ventral lobes or lips, which exhibit slight median emarginations, but are otherwise entire. It has sensory hairs and papillae, which are common in this family. The fourth annulus is very narrow.

The middle two appear as muscular rings. The chitinous jaws are triangular, the dorsal with a single tooth, the ventral jaw with a pair of smaller teeth. No lateral mucous glands which are very common in some of the species are present.

The body proper consists of eight strongly bi-annulate somites or rings. The anterior somites are longer and broader than the posterior. When contracted, the minor annuli of the somites are telescoped within the major annuli. The fifth, sixth, and seventh somites are sexual. The first, second, third, fourth and eighth somites are nephridial. The spermatheca is broad, thin walled, and nearly cylindrical. The penis is carried to the exterior by the eversible bursa, into which its projecting end is received. There is a conspicuous prostate in addition to the large glandular sperm sac. These parasitic forms remain attached to the ventral surface of the host throughout their entire life history. The eggs are deposited on the ventral surface of the host, more abundantly where the water is kept in constant motion by the movement of the appendages. Each egg is enclosed in a distinct separate stalked cocoon. The base of the stalk is firmly attached to the host. The deposition of eggs occurs during the entire year, if the parasites be kept in aquaria at room temperature. In their natural habitat eggs are not deposited during the severe winter months.

BRIEF OUTLINE OF DEVELOPMENT

The cleavage of the ovum takes place with considerable precision and regularity. Especially is one impressed with this striking phenomenon, after following the cleavage of many ova. The only perceptible variations being (a) slight differences in the time at which the individual cells divide; (b) slight variations in the size of the same cells in different ova. The rate of cleavage varies somewhat with temperature. Occasionally all the cleavage cells of an individual egg are nearly equal and it is impossible to orient the embryo before the germ bands begin their formation. This, however, is an exception, rather than a usual occurrence.

As development progresses the variations between individual embryos become less apparent and as far as can be recognized, do not affect the final result.

The history of the cleavage is distinguished by three well marked periods, namely: oblique, transitional, and bilateral. In the first period, which extends to the twenty-four-cell stage, the germ layers are differentiated, and the parent cells, which give rise to the future organs are definitely marked out.

The first cleavage is nearly transverse to the median longitudinal axis of the adult worm. The second cleavage plane occurs at an angle of forty-five degrees to the first. The third cleavage plane is horizontal and separates the four ectomeres above from the four macromeres below.

Three generations of four ectomeres each are successively separated from the macromeres A, B, C and D. The first generation of ectomeres (a^1 , b^1 , c^1 and d^1), are formed in a right handed direction. The second generation of ectomeres (a^2 , b^2 , c^2 and d^2), are formed in a left handed direction. The third generation of ectomeres (a^3 , b^3 , c^3 and d^3), are formed in a right handed direction. From these twelve ectomeres the entire ectoderm is formed.

The ectomere d^2 gives rise to all or nearly all the ectoderm of the trunk, to the nervous system and to the nephridia.

The oblique type of cleavage is maintained in the division of macromeres. At the close of the oblique period the embryo consists of twenty-four cells (text fig. 6 and fig. 36). The relation of the cleavage cells to the germ layers is as follows:

4.....	macromeres.....	entoderm.....
1.....	mesomere.....	mesoderm.....
20.....	ectomeres. {	19.....ectoderm
		1 (d^2)..ectoderm, nervous system, nephridia

Bilateral division now occurs in some of the ectomeres, while others may continue to divide obliquely. The transitional period shows both types of cleavage. Oblique cleavage persists in some of the cells until the fiftieth or more cell stage.

In the third period, the cleavage becomes essentially bilateral

in most of the cells and the teloblasts of the right and left halves of the embryo are formed. Bilateral symmetry now becomes definitely established and the animal increases in length very rapidly.

CLEAVAGE

1. DESIGNATION OF CLEAVAGE CELLS

In the designation of the cleavage cells, for the sake of uniformity and convenience, I have for the most part adopted the system followed by Wilson in his "Cell lineage of Nereis," and Lillie in his study on "The embryology of the Unionidae." The first four cells (macromeres) are designated by the capital letters A, B, C and D. The generations of micromeres (ectomeres) by the small letters a, b, c, and d. The first index number indicates the generation to which the ectomere belongs. Thus a^1 , $b^{1,2}$ or $c^{1,1,2}$ or $d^{1,1}$ all belong to the first generation; c^2 , $b^2, d^{2,3}$ belong to the second generation, etc. A, B, C and D correspond to the vegetal pole; a, b, c and d to the apical pole. When a cell divides the products receive the designation of the parent cell with the addition of a further index number; thus $b^2 \begin{cases} b^{2,1} \\ b^{2,2} \end{cases}$.

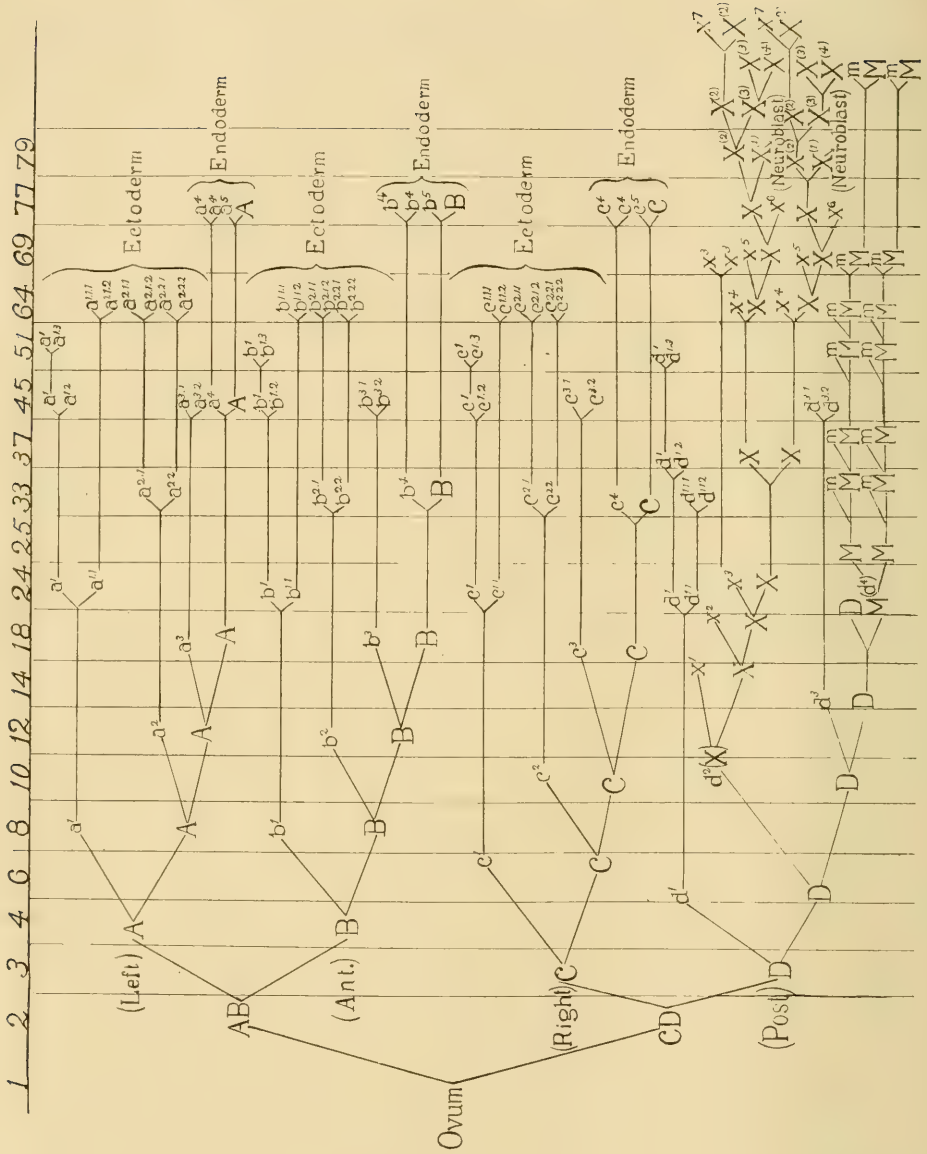
Exceptions to this rule are made only in the case of special cells, which, for convenience, receive special designations: thus d^2 of the second generation of ectomeres becomes the 'first somatoblast' and is designated by (X), and its small derivatives by x^1 , x^2 , x^3 , etc.; d^4 the 'second somatoblast' is designated by (M).

2. TYPES OF CLEAVAGE

a. The oblique period of cleavage: one to twenty-four cells

First cleavage: The first cleavage occurs about five to ten hours after the deposition of the egg. The time varies somewhat with external conditions. The plane of division passes through the area where the polar bodies are formed (fig. 1) and divides the egg into two very unequal parts, AB and CD (text fig. 1 and fig. 2). The smaller of the two cells AB is anterior, and

TABLE 1
Cleavages



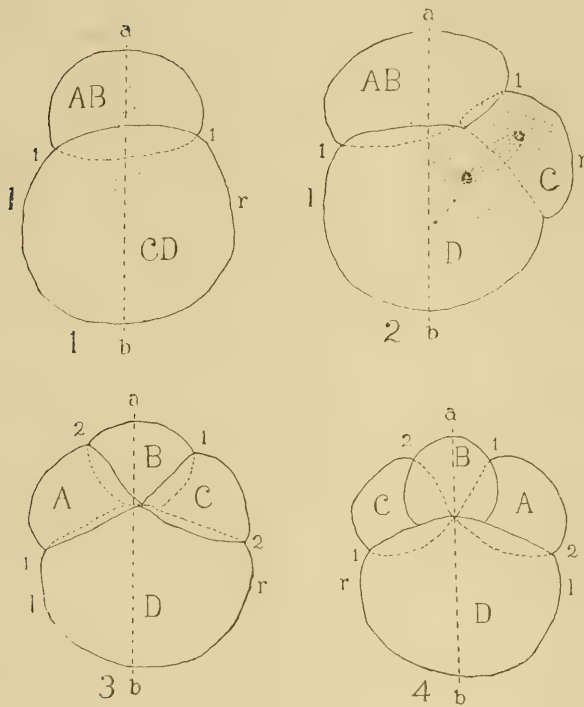


Fig. 1 Two-cell stage from apical pole view.

Fig. 2 Early three-cell stage from apical pole

Fig. 3 Four-cell stage from apical pole view.

Fig. 4 Four-cell stage from vegetal pole.

a-b, median longitudinal plane of future adult; *1-1*, first cleavage plane; *2-2* second cleavage plane; *a*, anterior; *b*, posterior; *r*, right side; *l*, left side.

the larger cell CD is posterior. The cleavage at first is very deep and the cells are rounded, but soon they begin to press against each other and flatten at their point of contact. Before the second cleavage begins the egg assumes its original elliptical shape and the point of contact externally, between the two cells is represented by a mere line or shallow groove. No actual fusion of the two cells ever takes place; sections always show a distinct line of separation between them.

The deutoplasm is equally distributed in both cells. The cytoplasm surrounding the nucleus contains very little yolk

material. This makes it possible, not only to recognize the position of the nucleus, but to be able to make out the exact position of the cleavage spindle in the living egg.

Second cleavage: The second cleavage is meridional and takes place at an angle of forty-five degrees to the median plane of the future adult. The two cells divide at different times (occasionally both cells divide simultaneously). These two cleavages taken together represent the second cleavage in other annelids. CD divides first into two very unequal parts (text fig. 2 and figs. 3, 78). The division of AB is nearly equal (figs. 5 and 6). The largest cell, D, is posterior. B is anterior, inclined a little to the right. C is right (text figs. 3-4) and A is left with reference to the median axis of the future worm. The large cell D has a tendency always to divide first. The exact formation of the four macromeres must be carefully worked out, and correctly understood, since their position largely determines the orientation of the future organs.

For descriptive conveniences the region of the first generation of ectomeres will be considered as the upper or apical pole and the point directly opposite, as the lower or vegetal pole. The centers of the upper and lower poles of the dividing ovum, coincide with the median longitudinal plane of the adult worm. The poles however may be shifted somewhat anteriorly or posteriorly, with reference to the macromeres, more especially to D in the formation of d^2 . When viewed from the upper pole A and C are in contact, while B and D are separated. But when viewed from the ventral pole A and C are separated and B and D are in contact (figs. 6-7). This extensive cross furrow found at the vegetal pole is also present in forms like *Nereis*, *Clepsine* and *Crepidula*; while in those forms like *Unio*, in which the greatest mass of the four macromeres is concerned in the formation of ectoderm instead of endoderm, the cross furrow is greatest at the animal pole. These cross furrows ('Brechungslinie' of Rauber) have no special significance in the development of *Bdellodrilus*, as the cleavage of the macromeres is carried to the end, immediately after the three generations of ectomeres are formed. In those forms like *Nereis*, where the cross furrow

DESCRIPTION OF PLATES

All drawings were made with a camera lucida under a magnification of about 125 diameters. All whole amount drawings, with one or two exceptions, were made from the living egg. The variation in size of the surface views is due to a difference in the size of the eggs. The sections were not uniformly magnified. Stippling has been adopted for the sake of clearness.

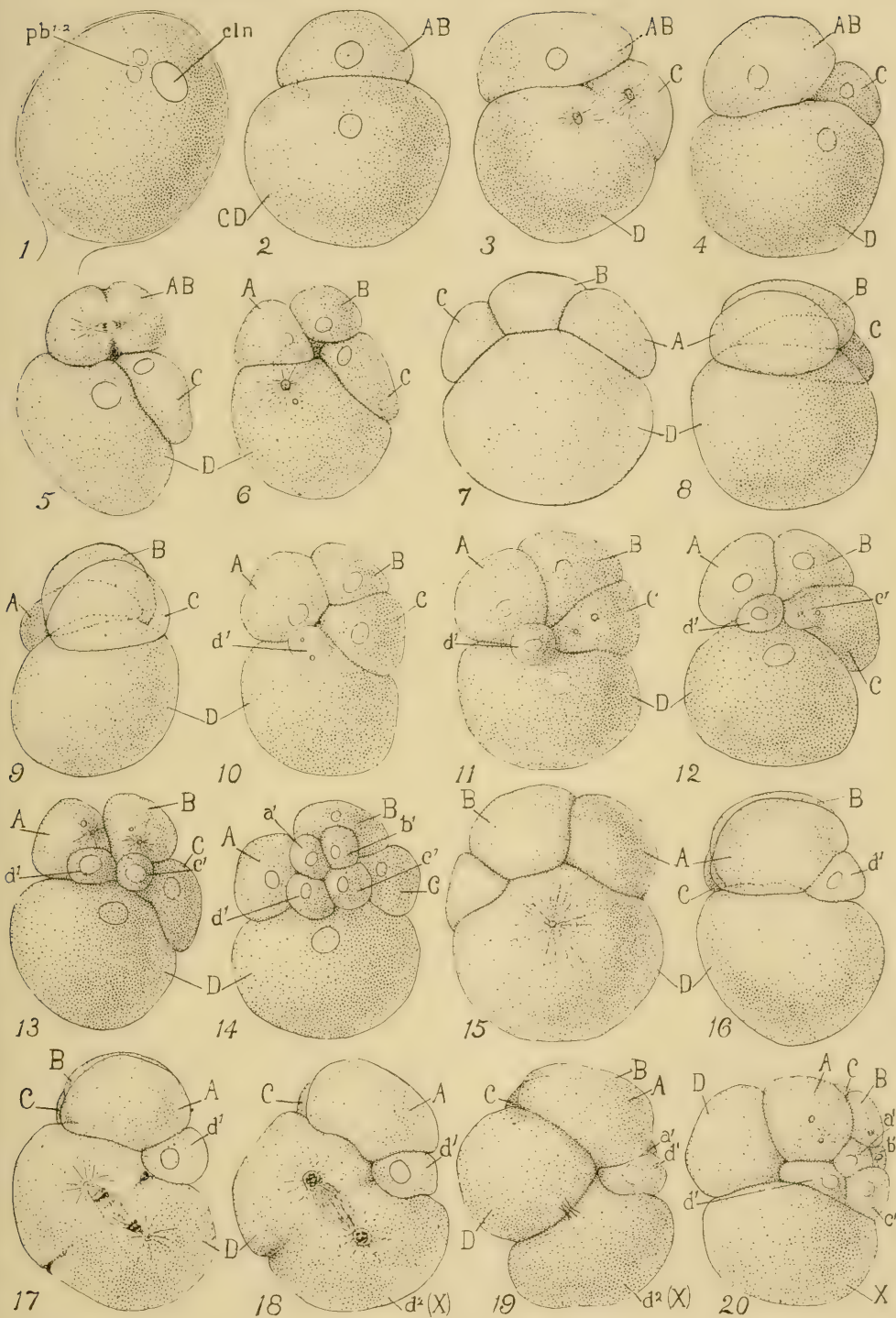
REFERENCE LETTERS

<i>a.</i> , anterior	<i>mi.a.</i> , minor annulus
<i>bl.</i> , blastopore	<i>mj.a.</i> , major annulus
<i>c.</i> , ciliated cells	<i>mo.</i> , mouth
<i>c.c.</i> , cleavage cavity	<i>p.</i> , posterior
<i>c.l.</i> , cerebral lobes	<i>pb¹⁻²</i> , polar bodies
<i>coe.</i> , coelom	<i>ph.</i> , pharynx
<i>ecp.</i> , egg capsule	<i>pr.</i> , proctodaeum
<i>ec.</i> , ectoderm	<i>so.mes.</i> , somatic mesoderm
<i>en.</i> , entoderm	<i>sp.mes.</i> , splanchnic mesoderm
<i>gn.</i> , ganglia	<i>st.</i> , stomodaeum
<i>mes.</i> , mesoderm	<i>v.</i> , ventral
<i>A</i> , left macromere	
<i>B</i> , anterior macromere	
<i>C</i> , right macromere	
<i>D</i> , posterior macromere	
<i>a¹</i> , <i>b¹</i> , <i>c¹</i> , <i>d¹</i> , <i>a^{1.1}</i> , etc., first generation of ectomeres	
<i>a²</i> , <i>b²</i> , <i>c²</i> , <i>d²</i> , <i>a^{2.1.1}</i> , etc., second generation of ectomeres	
<i>a³</i> , <i>b³</i> , <i>c³</i> , <i>d³</i> , <i>a^{3.1}</i> , etc., third generation of ectomeres	
<i>a⁴</i> , <i>b⁴</i> , <i>c⁴</i> , <i>d⁴</i> , etc., fourth generation of micromeres	
<i>X</i> = <i>d²</i> first somatoblast	
<i>X</i> , <i>X</i> , right and left proteloblasts	
<i>X⁽¹⁾</i> , neuroblast	
<i>X⁽²⁾</i> , <i>X⁽³⁾</i> , <i>X⁽⁴⁾</i> , nephroblasts	
<i>M</i> = <i>d⁴</i> second somatoblast	
<i>m</i> , secondary mesoblast	
<i>x^{1-x7}</i> , small derivatives from <i>X</i>	
<i>N</i> , posterior end of nephric rows	
<i>Nc</i> , posterior end of neural rows	
<i>nc</i> , neural rows	
<i>np</i> , nephric rows	

PLATE 1

EXPLANATION OF FIGURES

- 1 Surface view of an unsegmented ovum, to show the polar bodies and the cleavage nucleus.
- 2 Two-cell stage from the upper pole.
- 3 Two-cell stage; cell CD dividing.
- 4 Three-cell stage from the upper pole; division of CD is complete.
- 5 Same stage as preceding; cell AB dividing.
- 6 Four-cell stage from the upper pole; the cleavage spindle for the first ectomere forming.
- 7 Four-cell stage, ventral view.
- 8 Four-cell stage viewed from the left side.
- 9 Same as the preceding, viewed from the right side.
- 10 Four-cell stage from the upper pole, showing the formation of the first ectomere.
- 11 Five-cell stage from the upper pole, d^1 formed.
- 12 Stage showing the cleavage spindle of the second ectomere.
- 13 Six-cell stage from the upper pole; cleavage spindles for third and fourth ectomere forming.
- 14 Eight-cell stage from the upper pole; the macromeres are considerably flattened.
- 15 Same as the preceding, from the ventral pole; similar to the same view of the four-cell stage.
- 16 Eight-cell stage from the left side.
- 17 Same as the last showing the behavior of the macromere D in the formation of the first somatoblast.
- 18 Stage a little later than the preceding.
- 19 Nine-cell stage from the left side after the formation of the first somatoblast.
- 20 Nine-cell stage turned a little to the left, so that all the cells are visible.



persists until late development, it serves as an unmistakable point of orientation.

Figures 8 and 9 show the four-cell stage from the left and right sides. The dorsal ventral axis of A, B and C is about the same as that of D, but immediately after the formation of the first generation of ectomeres, the cells A, B and C shorten and become more rounded (fig. 16). In later stages of development these cells often become very much flattened and cause the developing embryo to appear unusually large, when viewed from the upper or lower poles.

Third cleavage (eight-cell stage): In the formation of the first generation of ectomeres (d^1 , c^1 , b^1 and a^1), each of the four macromeres divide obliquely. The ectomere end of the cleavage spindle is uppermost. The macromere D divides first; d^1 is budded off from D towards the upper pole, in the direction of the hands of a watch (dextrotropic), (figs. 10–11). We have thus a five-cell stage. Each of the macromeres C, B and A next bud off a small cell towards the upper pole. These are not formed simultaneously, but in the invariable order c^1 , b^1 and a^1 . Thus there occurs successively, a six, a seven and an eight-cell stage (figs. 11–14). In figure 13, an upper pole view, D and C have divided and A and B are preparing to divide. In both A and B the asters of the ectomere end of the spindles are visible. The position of the opposite end of the spindles are indicated by circles. This figure shows the oblique nature of the cleavage spindles. The spindle in A points to the space between A and B. The spindle in B points to the space between B and C. In figure 14, an eight-cell stage, the exact relation of the ectomeres and macromeres are shown as they normally appear from the apical pole. The position of the first generation of ectomeres is obvious. They suggest a possible rotation, after their formation, through an angle of about forty-five degrees in the direction of the hands of a watch. If actual rotation did occur there would be no difficulty in explaining their final position. But the fact that the cleavage spindles are oblique and the position of the completely divided nucleus can be definitely determined, before there is any indication of the cytoplasmic division of the

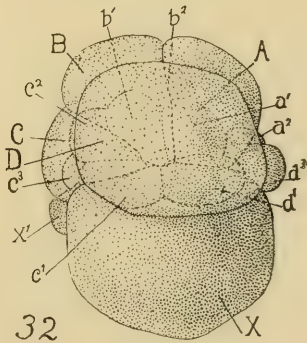
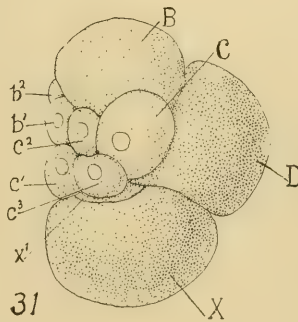
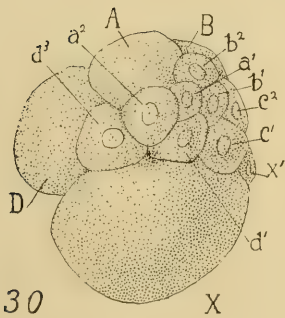
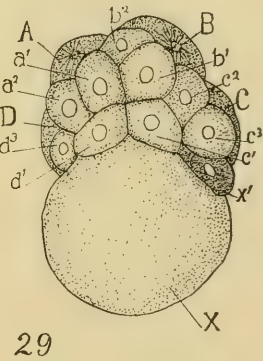
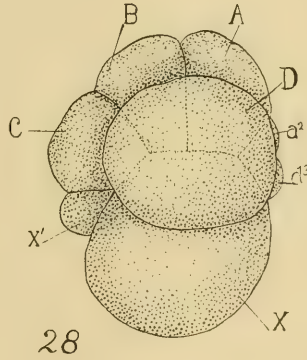
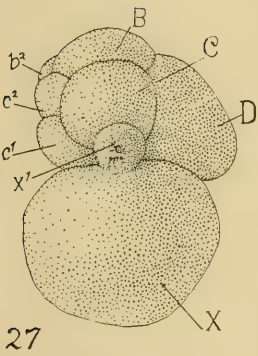
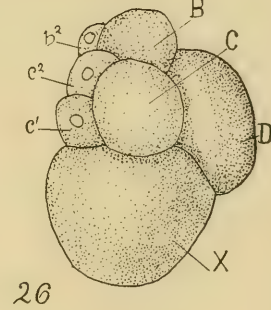
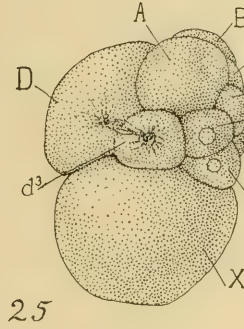
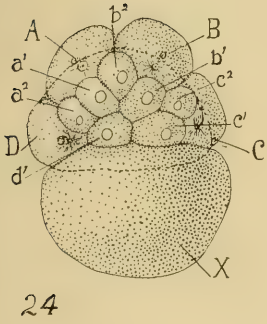
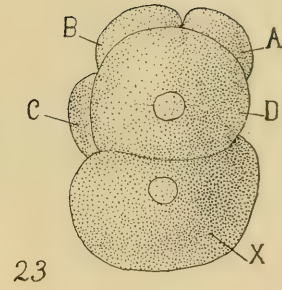
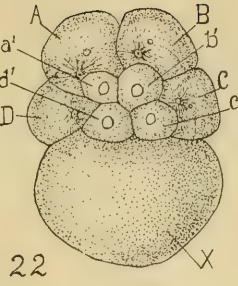
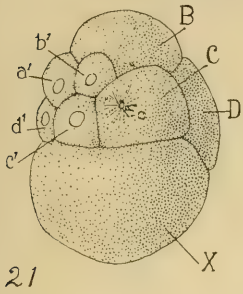
parent cell in the formation of the daughter cells, suggest that the apparent rotation process is not mechanical or even produced by pressure of the macromeres. The formation of d^1 in figure 10 shows how the process takes place; d^1 is budded off obliquely from the macromere D over the inner posterior edge of A and becomes partly imbedded in A. Its final position is determined by the direction of the cleavage spindle. This characteristic method in the formation of the ectomeres is quite a prevalent one. It occurs not only in the eggs of annelids, but in those of the molluscs and polyclads as well.

Fourth cleavage: A nine-cell stage is reached in *Bdellodrilus* by the division of the macromere D in an oblique direction. Figure 16, an eight-cell stage viewed from the left side, shows the position of the macromeres A, B and C with reference to the macromere D, before the formation of the ectomere d^2 . The large macromere D contains about two-thirds of the volume of the dividing ovum. In preparation for the formation of d^2 , D elongates in an oblique direction at an angle of about forty-five degrees to the horizontal plane of the developing embryo. The ventral anterior portion of D shifts forward beneath A, B and C (fig. 17). After the formation of d^2 , D takes a position directly beneath the first generation of ectomeres, and completely covers the inner ends of A, B and C (figs. 19-20). In some instances D is shifted more anterior and completely covers the ventral surface of the other macromeres (fig. 20); but in most cases, as in the nine-cell stage, D occupies the region of the ventral pole, directly beneath the first generation of ectomeres (figs. 22-23). The formation of d^2 is shown in figures 16 to 19. The division is equal in most cases. When unequal, d^2 is the larger cell. In figure 20, a nine-cell stage turned to the observers left so that all the cells are visible, A and B are preparing for the formation of a^2 and b^2 . In most the succeeding stages d^2 , the 'first somatoblast' will be designated by the capital letter X. It is also the first cell of the second generation of ectomeres. The formation of a^2 , b^2 and c^2 is shown in figures 20, 21, 22 and 24 in side and top views respectively. The second generation of ectomeres, with the exception of d^2 (X), is about the same size as those of the

PLATE 2

EXPLANATION OF FIGURES

- 21 Nine-cell stage, viewed from the right side.
- 22 Same stage as the preceding, from the upper pole; spindles for the second generation of ectomere are forming.
- 23 The same stage from the ventral pole.
- 24 Twelve-cell stage from the upper pole; spindles for the third generation of ectomeres are forming.
- 25 Thirteen-cell stage from the left side; the cell d^3 nearly formed.
- 26 Same stage as the preceding from the right side; the embryo is considerably elongated.
- 27 Fourteen-cell stage from the right side; X dividing to form x^1 .
- 28 Same stage as the preceding, ventral view.
- 29 Fifteen-cell stage from the upper pole; c^3 budded off symmetrical with d^2 .
- 30 Fifteen-cell stage from the left side; upper pole turned considerably to the left.
- 31 Same stage as the preceding from the right side; the small cell x^1 is drawn out between c^3 and X.
- 32 Fifteen-cell stage, ventral view, as a transparent object, with the position of all the cells indicated; drawn from a prepared specimen, cleared in xylol.



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first. The order of their formation is d^2 (X), c^2 , b^2 , a^2 , the same as the first generation. Figure 24 illustrates the twelve-cell stage from the upper pole. The cells are somewhat flattened. The macromere D is located a little to the left of the median longitudinal plane, while X is symmetrical with reference to the median axis of the future worm.

The cleavage spindles of the third generation of ectomeres form in an oblique direction. The thirteen-cell stage is reached by the formation of d^3 . The manner in which d^3 is formed, is rather unique when we take into consideration the size and position of D with reference to the other macromeres A, B and C (figs. 23-25). It is budded off from the outer surface of D and takes up a position symmetrical with c^3 . The fourteen-cell stage is reached by the formation of x^1 (fig. 27), it is budded off from the median right side of X. Its final position is between X and C. Figure 28 shows the same stage as the preceding in ventral view, turned a little to the observer's right.

Immediately after the formation of x^1 the macromere C buds off c^3 , thus making a distinct fifteen-cell stage (fig. 29). The order of formation of the third generation of ectomeres is the same as the first and second. Figures 29, 30, 31 and 32 show the fifteen-cell stage in dorsal, left, right and ventral views respectively. In figure 33, a^3 and b^3 are formed and the first generation of ectomeres are preparing to divide; d^1 and c^1 divide first; at the same time x^2 is budded off from X, symmetrical with x^1 , between D and d^3 , thus making a twenty-cell stage (fig. 34). Figure 35 is a side view of a twenty-two-cell stage after a^1 and b^1 have divided. This figure represents an anterior posterior elongation of the embryo, which is a very common occurrence. The cells, taken as a mass, are very plastic and may assume different shapes. This peculiarity is only secondary and has no special significance. The cells become more spherical before division and flatten out somewhat after the division is complete.

The twenty-three-cell stage is reached by the formation of x^3 from the upper posterior side of X between c^1 and d^1 (fig. 36).

The division of the first generation of ectomeres is unequal and radial rather than oblique. From the twenty- to the thirty-

cell stage several types of cleavage are present; oblique, radial and bilateral. This period of variable cleavage will be designated as the transitional period.

b. The transitional period of cleavage: twenty- to thirty-cell stage

After the formation of x^3 there is a short inactive period and in many of the developing embryos, the cleavage furrows become very indistinct. Cleavage is again initiated by the formation of d^4 from the large macromere D. The cleavage is oblique and very unequal (text fig. 5 and fig. 85).

The smaller cell is almost completely hidden when first formed. It is budded off directly between A and B, near the ventral anterior surface (fig. 37). The smaller cell persists as D (entomere) and the larger cell d^4 or M becomes the 'second somatoblast.' After the formation of M the entire endoderm is contained within the entomeres A, B, C and D (figs. 37-38).

The germ layers are now distinctly separated and the embryo at this stage of development is composed of twenty-four cells (text figs. 6-7). Nereis at the same period of differentiation, shows thirty-eight cells. Unio (Lillie) at the time of the separation of the germ layers contains thirty-two cells. This difference is due, in case of Bdello-drilus to the lagging of division in the cells of the upper pole.

The composition of the embryo at the twenty-four-cell stage is as follows:

Entomeres.....A, B, C, D.....	4
Ectomeres.....of first generation.....	8
Ectomeres.....of second generation.....	4
Ectomeres.....of third generation.....	4
Mesoblast.....M.....	1
First somatoblast derivatives.....	3

Many of the cells during the transitional period have a definite shape and if separated from the cell complex, they could be readily recognized. The embryo at this stage of development is somewhat spherical (figs. 36-38). Immediately after

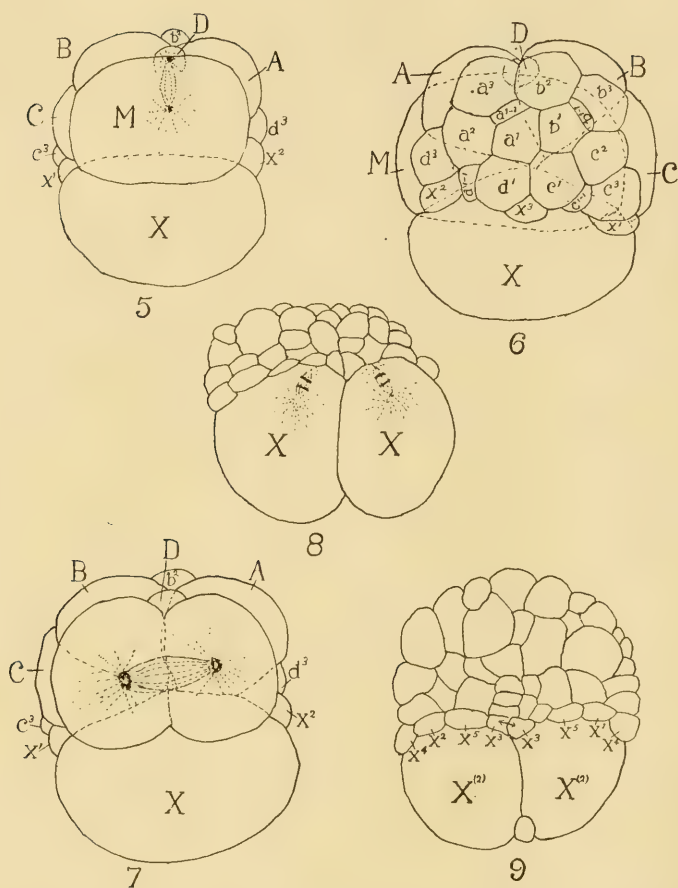


Fig. 5 Twenty-four-cell stage in ventral view, to show the division of the large macromere D. The larger of the two cells d^4 (M) becomes the 'second somatoblast.' The smaller cell D, becomes one of the four entomeres; D is scarcely visible from the exterior.

Fig. 6 Same as the preceding, in an apical pole view.

Fig. 7 Twenty-four-cell stage, ventral view, shows the bilateral division of M. D after the bilateral division of M becomes more distinct from surface view.

Fig. 8 Horizontal section of an early embryo to show spindles in the formation of x^5 from either proteloblast X, X.

Fig. 9 Horizontal section little later than preceding, to show the small cell x^1 - x^5 ; apical pole view.

the establishment of the germ layers, the bilateral division of the 'first and second somatoblasts' occurs. The bilateral division of the 'second somatoblast' usually precedes that of the first; occasionally they divide simultaneously.

c. The bilateral period of cleavage: twenty-five-cell stage

The first bilateral cleavages occur in the first and second somatoblasts (text fig. 7 and figs. 37-43). The small superficial cells of the lower pole are derived from the second and third generation of ectomeres and from the derivatives of X. The arrangement of these cells with reference to the blastopore is shown in figure 42. The entomeres A, B, C and D are partly grown over by the other cells and the open space becomes the blastopore. It is bounded anteriorly and laterally by small cells from the second and third quartettes, and posteriorly by the primary mesoblasts M, M. Its hinder lip, which is formed by the primary mesoblasts, lies anterior to the center of the lower pole. The closure of the blastopore takes place by a convergence of the cells from all sides. The principal growth of cells is from in front backwards, formed by the derivatives of the second and third generation of ectomeres (figs. 42, 43, 51). The entomeres now divide very rapidly and the cells soon become smaller than those of the ectomeres, which grow over them (figs. 43, 51).

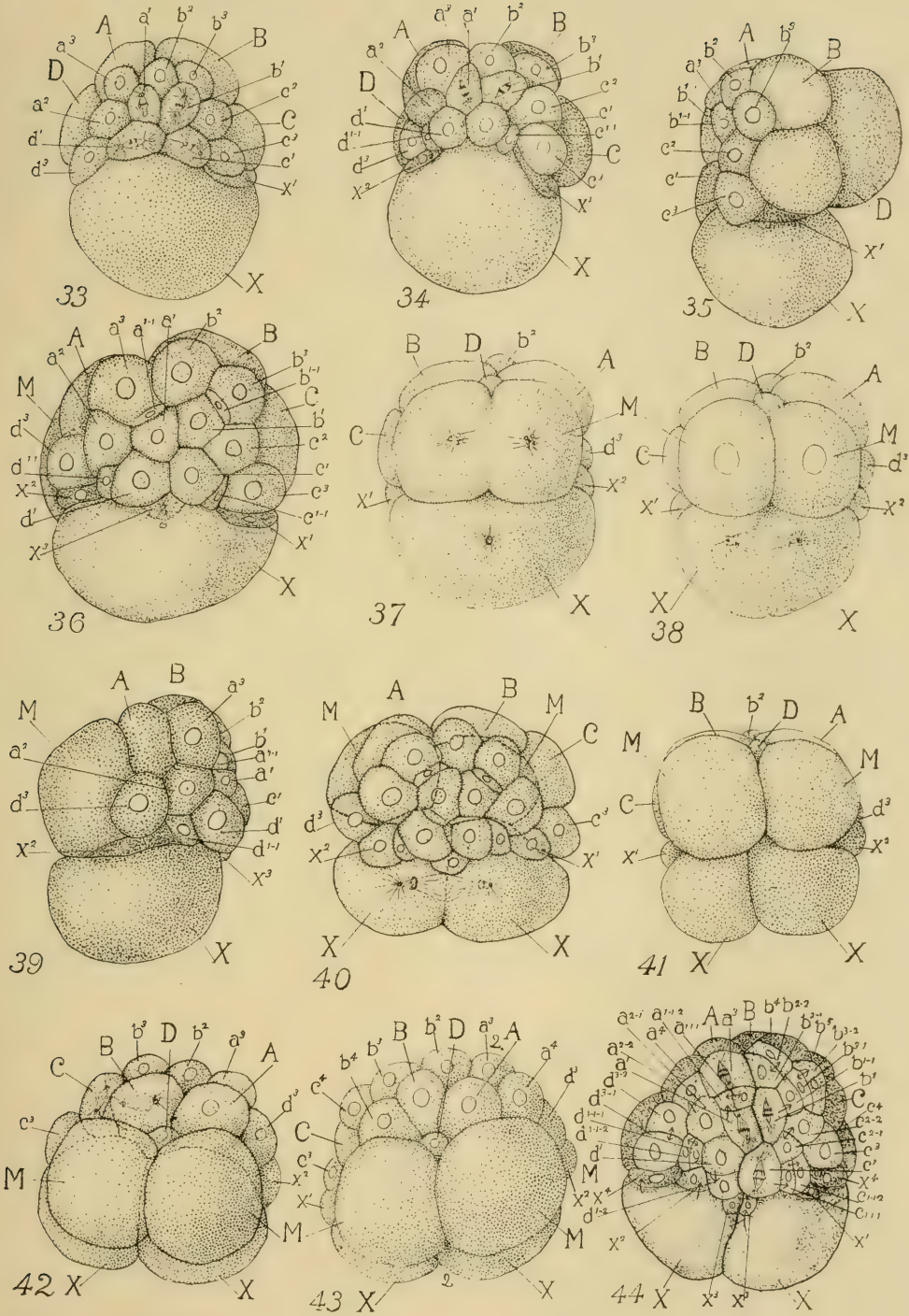
3. THE FIRST SOMATOBLAST

The history of the 'first somatoblast' in *Bdellodrilus* is of considerable interest when considered from the standpoint of its origin and its derivatives. When first formed from the posterior macromere D, it contains one-third of the entire bulk of the developing embryo. As already described, it first buds off the small cell x^1 on the right, x^2 symmetrically on the left and a third cell, x^3 , on the median posterior upper side. These three small cells are symmetrical with reference to the median longitudinal axis. The fourth cleavage divides the somato-

PLATE 3

EXPLANATION OF FIGURES

- 33 Seventeen-cell stage from the upper pole; spindles in the first generation of ectomeres forming.
- 34 Twenty-cell stage from the upper pole; cells c^{1-1} , d^{1-1} and x^2 just formed.
- 35 Twenty-one-cell stage from the right side; b^{1-1} , new cell; this embryo is unusually elongated.
- 36 Twenty-three-cell stage from the upper pole; d^{1-1} and x^3 , two new cells formed; embryo considerably flattened.
- 37 Twenty-four-cell stage from the ventral side; the unequal division of D has just occurred; D partially visible; the cleavage spindle of M forming.
- 38 Twenty-five-cell stage, ventral view; division of M complete and the spindle for the first bilateral division of X is forming.
- 39 Twenty-five-cell stage from the left side.
- 40 Twenty-five-cell stage from the upper pole; embryo is nearly spherical.
- 41 Twenty-six-cell stage, ventral view.
- 42 Same as the preceding, ventro-anterior view.
- 43 Twenty-nine-cell stage, same aspect as preceding; a^4 , b^4 and c^4 are the three new cells formed.
- 44 Forty-two-cell stage from the upper pole; increase in number of cells due to the rapid division of the ectomeres.



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PLATE 4

EXPLANATION OF FIGURES

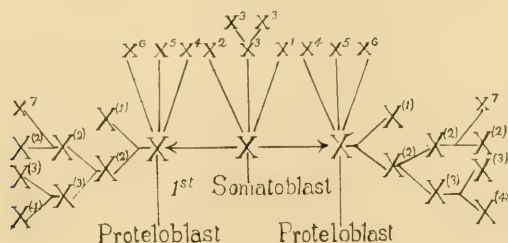
- 45 Sixty-three-cell stage from the upper pole.
- 46 Same stage as the preceding, from the ventral pole; very few of the ectomeres are visible.
- 47 Seventy-cell-stage, ventral view. Increase in number due to division of small cells. Consult table of cleavage. First nearly equal division of the proteloblasts, X, X. This division separates the neural and nephridial elements.
- 48 Seventy-two-cell stage, ventral view.
- 49 Little later than the preceding stage, to show x^6 and x^7 .
- 50 Embryo, ventral view, to show the first division of the nephroblasts; transverse axis greater than the longitudinal.
- 51 Embryo, ventral view, turned anteriorly to show the blastopore.
- 52 Embryo to show the lengthening of the anteros-posterior axis. The small cells, x^6 , x^6 , are good points to mark the orientation of the different figures. All figures on plate are similarly orientiated with reference to the right and left sides of the embryo.
- 53 Stage a little later than the preceding.
- 54 Embryo from upper pole, to show derivatives of x^6 between the nephroblasts.
- 55 Embryo with upper surface turned posteriorly, to show the rapid division of the ectoblast cells.
- 56 and 57 Show that either nephroblast $X^{(2)}$ or $X^{(3)}$ may divide, to form the three nephroblasts on either side.

blast into two equal parts, right and left (figs. 40–41). These two cells, for convenience in description, will be called the posterior right and left proteloblasts. At the fifth division each proteloblast buds off a small cell, x^4 , external to x^1 and x^2 respectively (fig. 44). At the sixth division each of the proteloblasts buds forth a small cell, x^5 on either side of x^3 , beneath the derivatives of c^1 and d^1 (text fig. 8). At the seventh division each of the proteloblasts buds off a small cell, x^6 on the ventro-anterior edge at the junction of the two cells (fig. 46).

At the eighth division each proteloblast, on either side of the median plane of the embryo, divided into two equal parts (figs. 47, 48, 93). The four cells formed become the posterior teloblasts, $X^{(1)}$ and $X^{(2)}$, right and left of the median axis (fig. 47). $X^{(1)}$ without any further division, becomes the neuroblast on either side, and $X^{(2)}$ becomes the nephroblast on either side of the median axis (figs. 47–48). Next $X^{(2)}$, right and left, divides equally, giving $X^{(2)}$ and $X^{(3)}$ (figs. 50, 52). Next either $X^{(2)}$ or $X^{(3)}$ divides equally. If $X^{(3)}$ divide, which is the common occurrence, we get $X^{(3)}$ and $X^{(4)}$. But if $X^{(2)}$ divide instead of $X^{(3)}$, the final result is the same. In either case, we get four teloblasts on each side (one neuroblast and three nephroblasts) as shown in figures 56 and 57. Next $X^{(2)}$ on either side divides very unequally and gives rise to x^7 on the anterior ventral outer surface (fig. 49).

The progeny of the ‘first somatoblast,’ when the teloblasts are completely formed, is twenty cells. Table 2 shows the derivatives of the ‘first somatoblast.’

TABLE 2



4. THE SECOND SOMATOBLAST

Immediately after the formation of X from the posterior macromere D, d^3 is budded off from D (figs. 24-25). Next D divides very unequally in an oblique direction and gives rise to d^4 (M) the 'second somatoblast,' as previously described. Figures 37 and 85 show the position of D and M after the cleavage of the macromere D. The twenty-five-cell stage is reached by the bilateral division of M (figs. 37-38). The cells M, M at first are a little to the left of the median plane, but later in course of development they become symmetrical to the longitudinal axis of the adult worm.

Soon after the bilateral division of M, the 'second somatoblast,' each cell M, M right and left buds off five or more small cells directly beneath the first generation of ectomeres (figs. 86-87). It is impossible to detect these cells except by means of sections, hence the uncertainty as to their exact number. They are characterized by their large nuclei with homogeneous staining chromatin and they contain but little yolk material. These small cells divide once or twice soon after their formation from the primary mesoblasts and then remain inactive until late embryological development, at least until after the germ bands are completely formed and the embryo has undergone considerable differentiation (as the formation of the lumen of the digestive system, etc.).

These undifferentiated mesodermal cells occupy the region which becomes the central dorsal side of the embryo, at the point where the developing worm is completely turned on itself (figs. 92, 98-99). The history of these cells can be readily followed through their different stages of development, so that there can be no question as to their exact origin and history. When the embryo begins to straighten, the progeny of these small cells extend toward either end and form the splanchnic and somatic mesoderm on the dorsal side of the worm. This secondary mesoderm later becomes continuous with the primary mesoderm, which forms directly from the mesoblasts M, M.

5. THE ENTOBLAST

The formation of the entoblast in *Bdellodrilus* represents an unusual type of development among the annelid worms. The macromeres A, B, C and D, after the formation of d^4 , give rise to the entire entoderm. D is greatly reduced after the formation of d^4 . The position of the entomeres is shown in figures 40 and 42. In figure 40 A, B and C appear rather large, because of the flattened condition of the cells. In figure 42 (from the ventral pole) the cells are rounded and appear more normal. The position of the entomeres and their boundary cells are distinctly shown. This figure shows more clearly the bulk of the entoderm, when compared with the mass of the entire egg. In figure 43 (a twenty-nine-cell stage) A, B and C have divided nearly equally. This division is considered by some investigators as the formation of the fourth generation of micromeres; d^4 of the D quadrant has formed earlier. Figures 44 and 45 (apical pole views) show the upper outer edge of the entodermal cells. In figure 46 (the same stage as preceding from the ventral pole) a very small part of the entodermal and ectodermal cells are visible. This figure shows the prominence of the four large cells, which later form the ten teloblasts. These four large cells, from their position, resemble the four large entomeres, which are so prominent in many other forms. These cells (X, X, M, M), according to Selensky, share equally in the formation of the germ layers, i.e., ectoderm, endoderm and mesoderm are produced by each of them.

In forms like *Clepsine*, *Crepidula* and others, at a similar or later stage of development, the entomeres are very prominent and the ectomeres with the first and second somatoblasts, form a cap of cells on their upper surface. In *Bdellodrilus* the conditions are different. The ectomeres and the entodermal cells form a cap of cells on the upper anterior surface of X, X, M and M. This difference is due to the prominence of the first and second somatoblasts, which constitute the greatest bulk of the embryo. At about the seventy-cell stage the ectodermal and endodermal cells are nearly uniform in size (figs. 47-49). In

figure 51 (a little later stage) the blastopore is nearly closed. This early closure of the blastopore in *Bdellodrilus*, is due largely, to the ventro-anterior shifting of the macromere D over A, B and C in the formation of the somatoblasts (figs. 18, 85).

The closure of the blastopore, in some of the annelids, occurs at a very late stage of development. In Clepsine the teloblasts give rise to rows of cells, which pass anteriorly around the entomeres A, B and C beneath the edge of the blastodisc or cap of cells. The blastodisc with these rows of cell cover about half of the entomeres. By the downward growth of the blastodisc and the concrescence of the germ bands, the closure of the blastopore is completed. The closure of the blastopore in Clepsine occurs on the ventral side, nearer the anterior end. In *Bdellodrilus*, the germ bands are not formed until later and take no part in the closure of the blastopore. Text figures 10 to 13 show the position of the ectoderm, entoderm, and the first and second somatoblasts, at different stages in the closure of the blastopore. The region of closure is similar to that of Clepsine.

At the time of the formation of the secondary mesoblast just beneath the first generation of ectomeres, the entire entoderm is situated in the anterior half of the embryo. But soon after the formation of the m cells (text fig. 12 and fig. 86), the entodermal cells by a rapid proliferation extend posteriorly between the m cells and the primary mesoblast. During the formation of the primary mesoblast, the meso-teloblasts themselves are carried posteriorly, ahead of the entoderm. The entoderm, thus becomes situated between the m cells or secondary mesoderm above and the mesoblast bands or primary mesoblast below. The entoderm in reality never reaches the posterior limit of the meso-teloblasts, as shown in figures 98 and 99.

The interior of the developing embryo, now consists of a solid mass of small entodermal cleavage cells (figs. 95-99), heavily laden with yolk. These cells are readily distinguished from the surrounding mesodermal cells, by their deeper cytoplasmic stain. Figure 99 (a vertical longitudinal section near the median plane) shows the position of the entodermal cells in the embryo.

As the embryo elongates, the entodermal cells increase in number. This process of growth is continued until the digestive tract is completely formed. Figure 99 shows the anterior and posterior limits of the digestive tract, which is formed from the four entomeres. The anterior end shows a distinct lumen, while the posterior end is yet a solid mass of cells. The entire digestive tract, except the insignificant stomodaeum and proctodaeum, is entodermal in origin. The proctodaeum is not formed until the time of hatching. It occurs on the dorsal side of the tenth segment. The embryo is completely turned on itself (fig. 99) and brings the anterior and posterior ends of the digestive tract in close proximity. The differentiation of the digestive tract begins anteriorly and progresses posteriorly. As growth continues, the outer cells of the entodermal mass form an epithelial layer. At first the cells are somewhat flattened, but soon take a columnar position, and form the columnar epithelium of the digestive tract. The cells within soon lose their staining properties, break up and serve as food for the developing embryo.

The digestive tract in its course of development, passes through the following stages: the first stage is represented by the four entomeres A, B, C and D; the second stage by a solid mass of cleavage cells (the cell boundaries are often very indistinct) within the center of the embryo; the third stage by an elongation of the entodermal mass as the larva lengthens, and by the establishment of a lumen; the fourth stage by a thin layer of flattened epithelium and later a columnar epithelium; the fifth stage, the cells within the epithelial layer serve as food; and sixth the formation of the stomodaeum and proctodaeum.

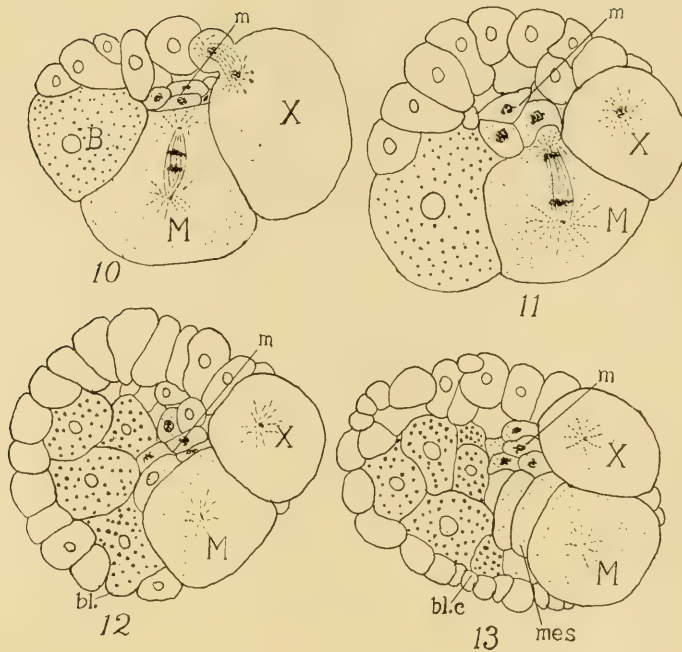
GENERAL HISTORY OF THE GERM BANDS

The term 'germ bands' has been variously interpreted by different investigators on cell lineage. The term germ bands, or the German equivalent 'Keim Steifen,' is usually restricted to the strata derived from the teloblasts, the ectoblastic layer being excluded. It is held by others that the germ bands of annelids are purely mesoblastic.

Balfour, Hatschek, Goette, Kowalevsky and many others made use of the term 'mesoblastic bands' as the equivalent of the germ bands. In Hirudinea, according to Whitman, the germ bands are composed of three distinct layers; the ectoblastic, mesoblastic and the neuroblastic elements. Wilson gave the same interpretation in his studies on "The embryology of *Lumbricus*." In *Bdellodrilus* the term 'germ bands' includes the three strata of cells as in Hirudinea and *Lumbricus*.

1. INNER STRATUM OF THE GERM BANDS

After the formation of the teloblasts, five on either side of the median axis (one neuroblast, one mesoblast and three nephroblasts), the mesoblasts or meso-teloblasts are the first to begin the formation of the germ bands by a forward proliferation of cells near the posterior lip of the blastopore (text figs. 12, 13, 17). The plane of division is nearly at right angles to the formation of cells in the secondary mesoblast (text figs. 10, 13). The cells of the mesoblast bands are considerably smaller than the teloblasts from which they originate. They grow forward between the entoderm and the ectoderm and finally meet at the anterior end of the larva. As these bands grow forward they become several cells broad, but seldom more than two cells deep. Their differentiation begins anteriorly and progresses backward. The first cells of the mesoblast bands, when formed, are on the surface, but soon become covered by the ectodermal cells. As the mesoblast band extends forward below and around the entoderm, it forces its way to the extreme anterior end of the embryo beneath the ectoderm. It finally encloses the digestive tract on the ventral and lateral sides and becomes continuous with the secondary mesoblast on the dorsal side. The two mesoblast bands fuse first at the anterior end along the median ventral side and subsequently with the dorsal secondary mesoderm. In figure 99 (a longitudinal section) the mesoblast is differentiated into splanchnic and somatic layers, with the coelom between. The longitudinal muscles become differentiated before the circular. At the extreme posterior end the



Figs. 10-13 Diagrammatic figures to show the ventro-posterior extension of the ectomeres, in the closure of the blastopore.

Fig. 10 Thirty-three cell stage, taken a little to the right of the median plane.

Fig. 11 Vertical section to the left of the median plane.

Fig. 12 Vertical section of a ninety-cell stage.

Fig. 13 Vertical section of an embryo at the time of closing of the blastopore. The mesoblast bands have just begun.

The heavy stippling represent endoderm; the light stippling mesoderm, and the unstippled the ectoderm or ectomeres. *m*, secondary mesoblast; *M*, mesoblasts; *bl*, blastopore; *bl.c*, point where the blastopore closes; *X*, derivatives of the 'first somatoblast'; *mes*, mesoblast bands.

meso-teloblasts are represented by an undifferentiated mass of cells, which later give rise to the musculature of the last three segments of the worm, and are directly concerned in the formation and movements of the posterior sucker.

2. MIDDLE STRATUM OF THE GERM BANDS

The middle stratum of the germ bands can readily be distinguished while the embryo is still nearly spherical. Upon close examination it is seen that the ectoblast cells are arranged into four distinct rows, on either side of the median ventral axis (figs. 65-66). Each row terminates posteriorly in a large cell or teloblast.

Text figures 15 and 17 and figure 58 show the early formation of these rows of cells. Sections of these various stages show that

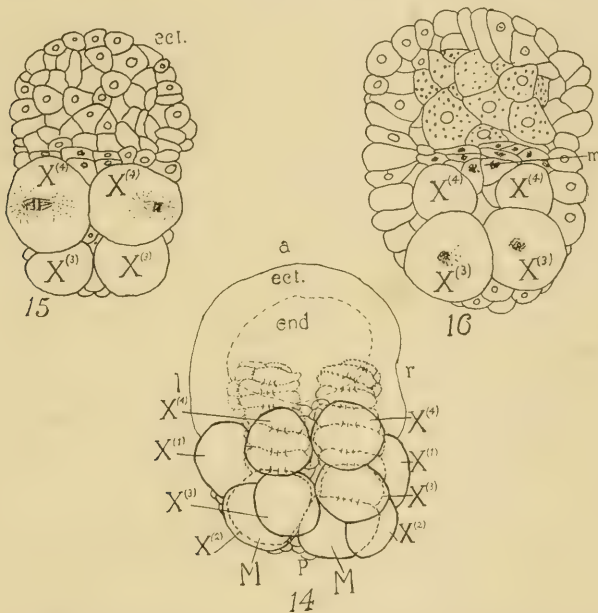


Fig. 14 Surface view from upper pole, to show the position of the ten teloblasts. The meso-teloblasts or mesoblasts have budded off eight or ten cells in the formation of the mesoblast bands. Their position is indicated by dotted outline. The broken outline represents the region of the entoderm. The position of the ectoderm is indicated by a continuous line.

Fig. 15 Third horizontal section from the top, passing through four of the large nephroblasts. The spindles represent the beginning of the first division in the formation of the middle germ band. The anterior end and the right side of the section are a little below the horizontal plane.

Fig. 16 Seventh horizontal section from the top. It passes through the upper portion of the entoderm and the secondary mesoblast (m).

these rows of cells form a part of the general ectoderm, being partly covered here and there by adjoining cells. In later stages of development, these rows of cells become completely covered as they gradually sink beneath the surface, and thus come to lie between the mesoblast and the ectoderm or ectoblast.

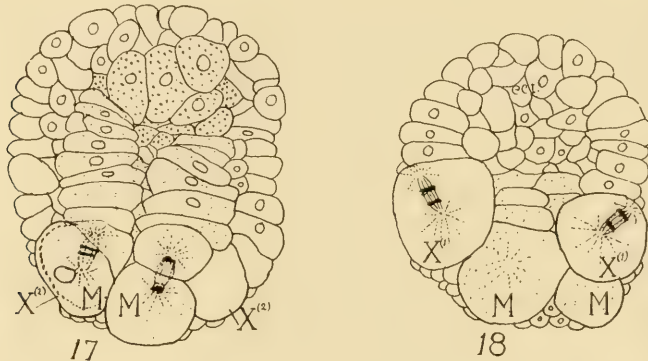


Fig. 17 Twelfth section from the top, to show the anterior extension of the mesoblast bands below and around the entoderm.

Fig. 18 Nineteenth section from the top, to show the lower side of the mesoblast. The section passes below the entoderm. The spindles represent the beginning of the first division of the neuroblasts to form the neural rows.

Heavy stippling represents entoderm; light stippling mesoderm and the unstippled portion the ectoderm. The sections of figures 15-18 were eight micra thick. There were 21 sections in all. *a*, anterior, and *p*, posterior, represent the respective ends of the cleavage cells, but not the future ends of the embryo; *r*, right; *l*, left; *ent*, entoderm and *ect*, ectoderm.

3. OUTER STRATUM OF THE GERM BANDS

This stratum forms the definitive ectoderm and needs no further description at this point of development.

The embryo now elongates very rapidly, and the general shape of the adult worm becomes recognizable. The teloblasts become less and less distinct, until finally the cell rows terminate posteriorly in a group of small cells. The mesoteloblasts extend farther posteriorly than the neuro-teloblasts. New cells are always formed from the anterior surface of the teloblasts. There can be no question as to the origin of the germ bands from corresponding teloblasts, as their formation

can be followed step by step. The mesoblastic and neuroblastic portions of the germ bands can be traced to the anterior end of the embryo. The meso-teloblasts are the last to disappear. They are distinct until after the formation of the stomodaeum and its connection with the pharynx. The concrescence of the germ bands begins anteriorly and progresses posteriorly.

THE ECTODERM AND ITS PRODUCTS

The three generations of ectomeres are given entirely to the formation of the ectoderm, which later becomes differentiated into the definitive hypodermis, with its glands, the cuticle and the anterior and posterior ends of the digestive tract. The ectoderm includes, in addition to the above, all of the teloblasts, except the two larger and deeper ones which represent the mesoblasts. The reason for regarding the eight teloblasts and their derivatives as a part of the general ectoderm, is on account of their origin and position. In position, they are superficial at first and can not be distinguished from the general ectoderm, except by their arrangement in rows. Small cells are budded off from the teloblasts, which form the trunk ectoderm. In *Clepsine* these teloblasts are at first superficial at the posterior end of the embryo. In *Lumbricus* they are found directly in the general ectoderm, and beyond question form a part of it.

1. THE NERVOUS SYSTEM

The nerve chain in *Bdellodrilus* first appears as a double row of cells, nearly uniform in size. Each row of cells originates from a single cell, the neuroblast. The neuroblasts, when first formed, are widely separated, but symmetrical to the median axis of the body. Figures 47 and 48 (in ventral view) show their position when first formed by an equal division of the proteloblasts X and X. They take up their position on either side of the mesoblasts (figs. 47, 48, 93). When first formed the neuroblasts are turned somewhat anteriorly as shown in the horizontal section of figure 93. This movement of the cells to their final position, independent of the former position of the cleavage

spindle, is a common occurrence in *Bdellodrilus*. In some instances it is necessary to employ sections, in order to determine the origin of cells. The transverse axis of the embryo at this stage is often greater than the longitudinal (figs. 49–50). This condition persists for a brief period only, during the formation of the teloblasts. As the embryo increases in length the neuroblasts are carried more and more posteriorly (figs. 56–57).

In order to get a better understanding of the origin and orientation of the neuroblasts— $X^{(1)}$ right and $X^{(1)}$ left—with reference to the other teloblasts, the figures of plate 4 are so arranged that the left side of the developing embryo corresponds to the reader's left. In figure 45 the upper pole is turned a little posteriorly, to show the upper outer edges of the entodermal cells. Figure 47 (from ventral pole) shows some of the ectodermal cells. The remaining figures are either turned forward or backward on their transverse axes. The ectomeres x^6 and x^6 right and left serve as good points for orientation (figs. 46–53). After the formation of the teloblasts, bilateral symmetry is fully established. The meso-teloblasts, however in some instances, are still a little to the left of the median axis. This variation in the symmetry of the mesoblast does not in any way change the end result. In the early history of the germ band formation the teloblasts $X^{(3)}$ and $X^{(4)}$ are slightly separated, while $X^{(1)}$ and $X^{(2)}$ are widely separated from the corresponding teloblast on the opposite side (figs. 56, 58). The neuroblasts and the nephroblasts begin their proliferation of cells to form the germ bands, about the same time (fig. 58). At this stage of development, the exact orientation of the embryo is distinct. Since the embryo is completely turned on itself, the further use of the terms, apical and ventral poles, is significant only as being convenient in description. The mouth, as stated above, is formed in the center of the apical pole and the anus in close proximity on the dorsal side of the tenth segment. Figure 59 (upper pole view) shows the complete curvature of the embryo. The heavily shaded portion represents approximately, the boundary between the anterior and posterior ends. This figure shows that the teloblasts are coming more and more in a straight line. Since the two ends of

the embryo are in immediate contact, it is impossible, except by longitudinal sections, to determine the exact point of separation. The ectoderm of the anterior end of the embryo, which is derived from the three generations of ectomeres is continuous with the ectoderm derived from the 'first somatoblast.'

The separation of the two ends of the embryo becomes recognizable in the early formation of the germ bands, as shown in figures 59 and 60. The posterior and ventral shifting of the neuroblasts (figs. 58-60) continues until all of the teloblasts are in a direct line. The small cells between the teloblasts are derived from the first somatoblast. In viewing the embryo from the upper pole (which now corresponds more to the anterior and posterior ends of the future animal) the germ bands extend laterally, downward and forward, being curved somewhat posteriorly as they pass from the upper to the lower pole (fig. 59). The meso-teloblasts in figure 58 are still visible from the exterior. In figure 59 they are almost grown over, while in figure 60 they are completely covered. This is due to the posterior shifting of the neuroblasts and the growth of the ectomeres from above and below. In an embryo viewed from the right side (fig. 61, a little older than fig. 60), the position of the neural and nephric rows of the germ band are shown. As the rows extend anteriorly they are more difficult to distinguish from the ectoderm. The neural rows alone can be followed to the extreme anterior end. The posterior end of the embryo is widely blunt, while the anterior end is more rounded. The heavily shaded portion represents the point of separation between the two ends.

Figure 62 represents the same embryo from the upper pole, with the ends of the embryo rotated or turned a little posteriorly. In figures 63 and 64 (from right and left sides respectively) the embryo is more elongated and the point of separation between the two ends is more distinct. The neuroblasts are lagging in their posterior extension. Their position is median ventro-posterior, as shown in figure 65. Their concrescence is not yet complete at the posterior end. In the following stages of development the cells of the neural and nephric rows divide

PLATE 5

EXPLANATION OF FIGURES

58 Embryo from upper pole, tilted a little to the right. The position of the ten teloblasts are shown; the small cells between the teloblasts on the surface are derived from x^6 and x^7 on either side.

59 Same view as the preceding; the neuroblasts have migrated a little posteriorly and are approaching each other.

60 The ecto-teloblasts are nearly in a direct line; the germ bands have begun to form; the two primary mesoblasts M, M are no longer visible from the exterior; the transverse heavily shaded portion shows the approximate point of separation between the two ends.

61 Embryo viewed from the left side; the posterior end is extremely blunt.

62 Same embryo as preceding, from the upper pole (upper pole corresponds to the anterior and posterior ends). Shows very strikingly the close proximity of the two ends.

63-65 Represent the same embryo from the right, left and ventral sides respectively. The ectoderm which partially covers the germ bands is not shown.

66 Embryo from upper pole; bilateral symmetry is well marked; the teloblasts are considerably reduced by the time they come in contact with their fellows on the opposite side.

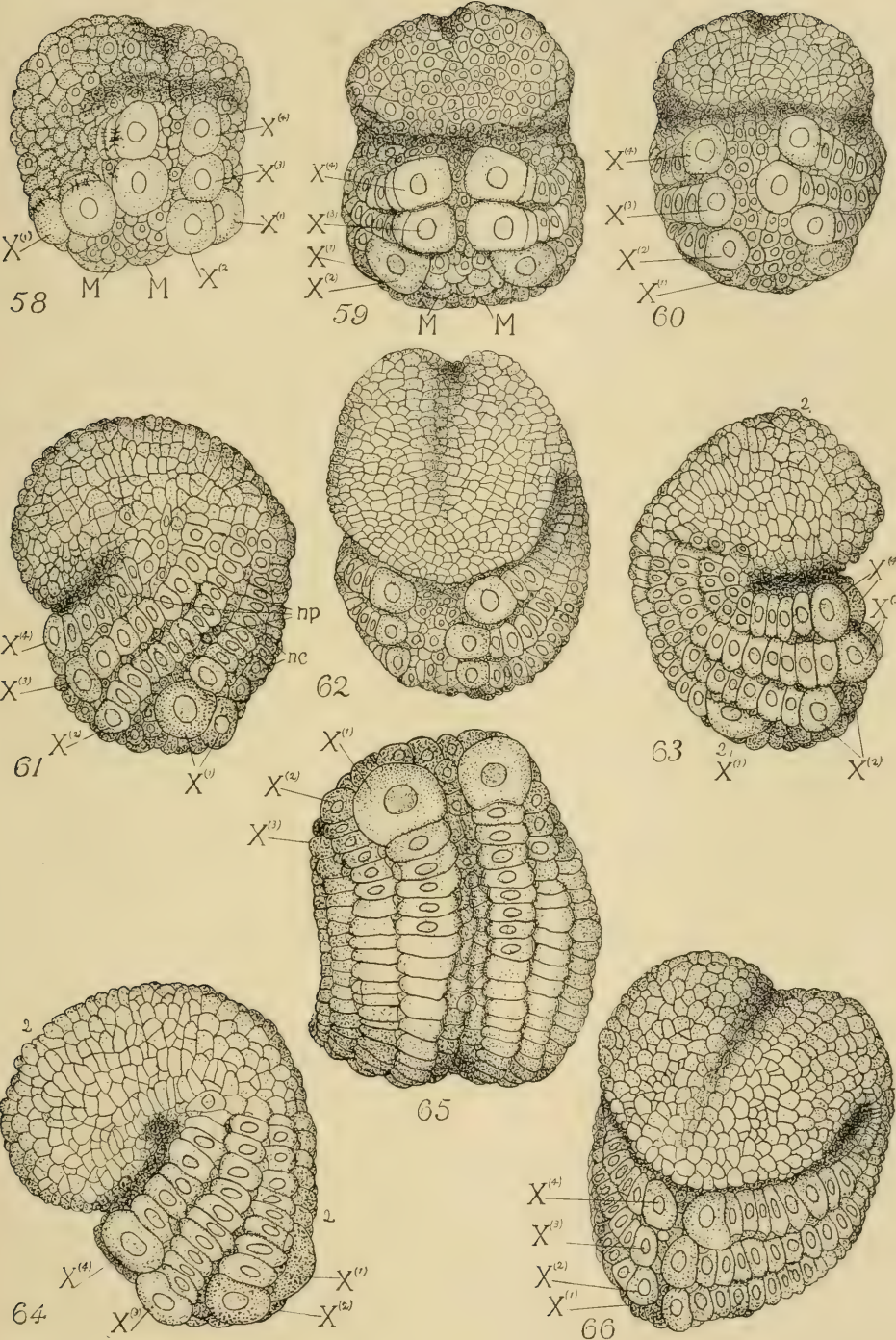


PLATE 6

EXPLANATION OF FIGURES

67 Embryo turned slightly to the left to show the anterior and the posterior ends; the embryo at this stage begins to rotate within the egg membrane.

68 The same as the preceding from the ventral pole, turned a little to the left.

69 Embryo viewed from the right side; condition before the posterior end becomes drawn out or pointed.

70 Embryo from the upper pole; shows compressed condition of the two ends; at this stage the embryo rotates very rapidly.

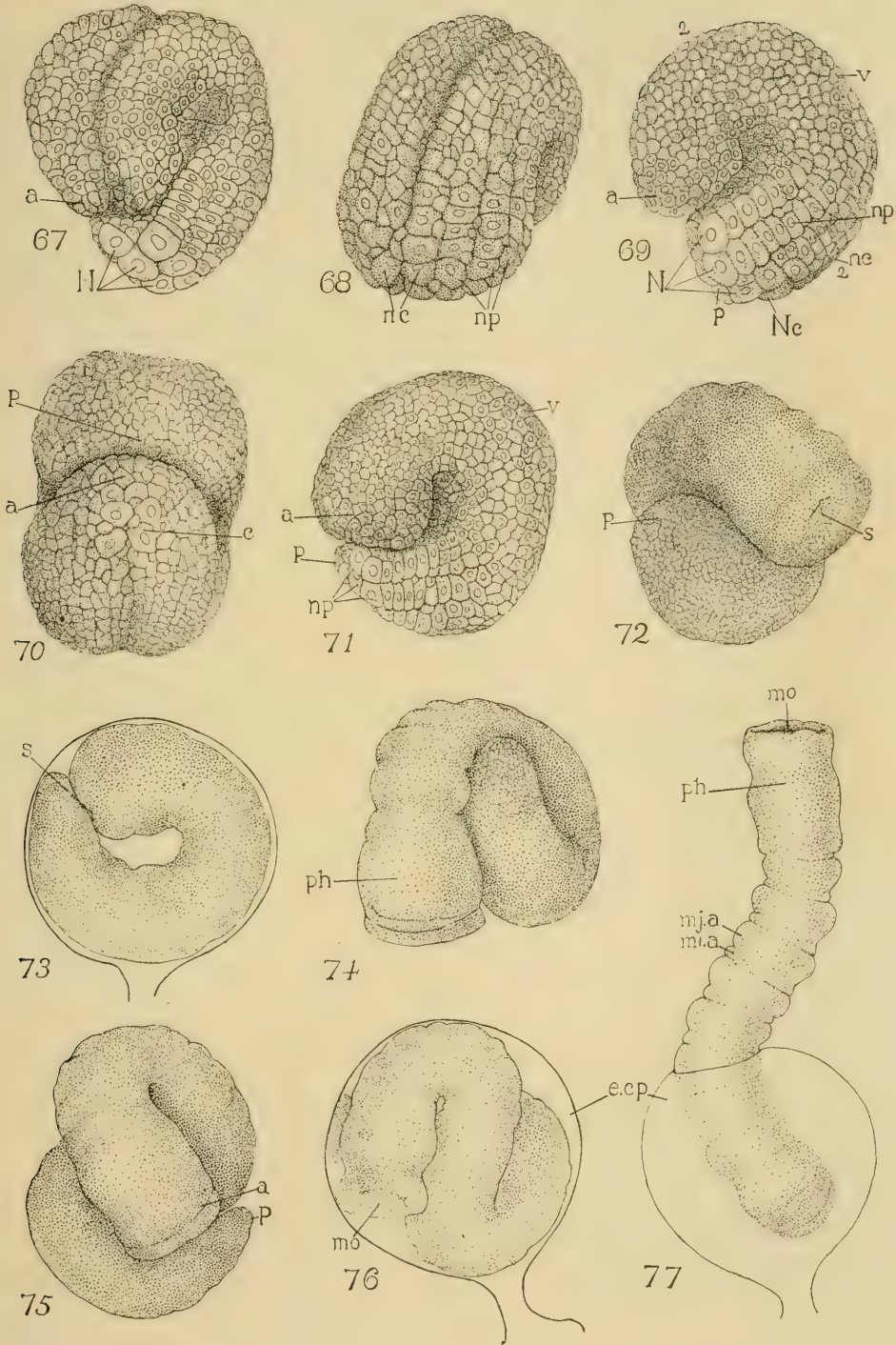
71 Embryo viewed from the right side; the teloblasts are partially visible at the posterior end; the tapering of the posterior end is well marked.

72 Embryo to show the overlapping of the ends; indications of segments are visible anteriorly; the stomadaeum is distinct.

73 Unusual condition, where the two ends remain in immediate contact until after the form of the worm is distinct; this occurs in eggs with an unusually large cocoon.

74-6 Different stages in the final growth of the embryo.

77 Condition of embryo at the time of emergence from the egg.



very rapidly and gradually become covered by the ectodermal cells as they sink beneath the surface (figs. 66-72), and form the middle stratum of the germ bands. In a nearly median longitudinal section (fig. 99), the relation of the parts are shown. The neural plate at different points shows the formation of ganglia. The anterior end of the section passes through the exact median plane and does not show any ganglia. The neuroblasts still persist at the posterior end.

The entire nervous system arises as two simple longitudinal rows of cells, and each row is produced by the continued proliferation of cells from a single cell, 'the neuroblast.' This proof is established by the study of surface preparations in connection with sections taken in different planes through the developing neural elements. The neural rows can be followed to the extreme anterior end, where they pass up around the pharynx and give rise to the cerebral ganglia on either side (fig. 96) by a thickening of the anterior extremity of the corresponding neural rows. There are exactly four rows of cells in the middle stratum of each germ band. The outline of the rows can be easily seen in surface views of the living embryo (figs. 63-66). They are more distinctly marked at the posterior ends, and become less distinct anteriorly, which is due to the more advanced development; that is, each row becomes double, then triple, etc. (figs. 68-69) and at the same time, its boundary lines become less distinct.

2. THE EXCRETORY SYSTEM

After the bilateral division of the 'first somatoblast,' each proteloblast contains the neural and nephric elements of their respective sides. According to Whitman, these two cells are called the neuro-nephroblasts. But when each proteloblast X , X divides equally the neural and nephric elements become separated, $X^{(1)}$ neural and $X^{(2)}$ nephridial (figs. 47-48). The nephroblast $X^{(2)}$ on either side next buds off a very small cell x^7 , which becomes ectodermal (fig. 49). Immediately after the formation of this small cell, $X^{(2)}$ divides nearly equally, and forms

$X^{(2)}$ and $X^{(3)}$ on either side (figs. 50-54). Both cells are nephridial. This fact perhaps is made more suggestive by the behavior of $X^{(2)}$ and $X^{(3)}$. Either of these cells may divide equally, but never both in the same embryo. In either case we have three teloblasts derived from the nephroblast $X^{(2)}$ on either side. The cells of the nephridial rows are somewhat smaller and narrower than those of the neural rows. In some cases the outer nephridial row of cells is very short. In other embryos it is composed of but one or two cells and its presence is hard to verify, suggesting a possible disappearance in the group. As stated above, the nephridia arise in connection with a continuous nephric cord of ectoblastic origin, which forms a part of the middle stratum of the germ band and lies along side of the neural row. Each nephric cord terminates at the posterior end in three teloblasts. Thus the entire nephric cord of cells is formed by the continued division of the 'nephroblasts,' which agree precisely with the neuroblasts in structure, action and mode of origin. The nephric cord at first is composed of three rows of cells posteriorly, but passing forward the rows are no longer definitely separated and the nephric cord or plate consists of an irregular series of cells which extend anteriorly to the posterior end of the pharynx. The formation of the nephridia progresses from in front backwards and keeps pace with the formation of new segments in the embryo. The beginnings of a pair of nephridia are found in each of the main segments. Only two pairs of nephridia are retained in the adult worm. The details of the formation of these segmental organs have not been worked out.

Berg considers the entire nephridia in *Criodrilus* as mesodermal in origin; Whitman held the extreme opposite view, that the entire nephridium was ectodermal in origin; while Wilson regarded the nephridia as being part mesodermal and part ectodermal in origin. In *Bdellodrilus* the nephridia are ectodermal. The anterior pair occurs in the first, second, third and fourth body segments. The left nephridia of the anterior pair, extends from the first to the third segments inclusive, while the right extends from the second to the fourth segments inclusive. Both

have a common opening on the dorsal side of the third segment. The funnel of the left occurs in the second and that of the right in the third segment. The posterior pair is found in the eighth segment. Each nephridium has a separate opening to the exterior on the dorsal side of the eighth segment.

GROWTH

The developing embryo does not increase appreciably in bulk until after the teloblasts are formed. Up to this period it is merely a division of the egg content into the various cell complexes. Even at this stage the increase in the long axis of the embryo is brought about by a decrease in the transverse diameter. Figures 50 and 55 show the transverse axis greater than the longitudinal, while in figure 56 and 57 the longitudinal axis is greater, due more to a change in shape than to growth. The egg content is very plastic and when removed from the cocoon the egg membrane, in most cases, is not of sufficient tenacity to retain the embryo intact. The ten teloblasts are shown in figures 56 and 57.

The first increase in length is due to the formation of the mesoblastic portion of the germ bands (text figs. 17-18). The neuroblastic and nephroblastic portions of the germ bands begin simultaneously after the meso-teloblasts have formed eight or ten cells (text figs. 15, 18 and fig. 58). Figures 58-71 show the various stages in the formation of the germ bands. Figure 71 is about the last stage when the germ bands can be detected externally. A longitudinal section of figure 71 near the median axis shows a differentiation of the germ bands into their incipient organs (fig. 99). From this point of development, growth is very rapid, and the embryo begins to rotate on its transverse axis. The movement is produced by the action of cilia, which occur on the large ectodermal cells in the median ventral half of the anterior end of the embryo (figs. 96-99). These cilia disappear before hatching, but the cells from which they are produced persist as a part of the ectoderm. The anterior and posterior ends are no longer in immediate contact, as in figure 71, but begin to overlap. The ends of the embryo

now take the position within the egg membrane of the least resistance to their further growth. Figure 74 shows the overlapping of the ends. The stomodaeum is completely formed and the annuli of the pharynx are visible. Figure 73 shows an unusual condition in the position of the ends. At this stage of development the animal often turns on its longitudinal axis, largely on account of the action of the muscles, and, instead of the convex side being ventral, it now becomes dorsal. This rotation on its longitudinal axis has no significance, as has been thought by previous investigators, in the later stages of development. The animal is extremely plastic and may assume any position or shape, as shown in figures 74 and 76. Figures 77 shows the completely developed animal at the time of emergence from the cocoon. The number of the segments is distinct. This peculiar form of growth within the cocoon is merely adaptive. Occasionally, when the cocoon is of an unusual size, the developing worm is less bent on itself.

A COMPARATIVE STUDY OF DIFFERENT FORMS

In following the cleavage cells of annelids, molluscs and polyclades, one is impressed with the striking resemblances in their different stages of development. If this marked similarity alone were a sufficient criterion for a basis of classification, some of the most widely separated forms, when considered from the standpoint of their early development, would be grouped as closely related species. How can such resemblances in development be explained in animals which are so unlike in their late stages of growth? Are they merely the result of such mechanical principles as surface tension, alternation of cleavage, and pressure, or is the nature and structure of the protoplasm the common cause? According to Driesch, 'the striking similarity' between the types of cleavage in annelids, molluscs and polyclades does not appear startling and is easy to understand, since cleavage is of no systematic worth. However, the more recent investigators on cell lineage, according to Heath, look upon the early cleavage stages as something more than a mere

manifestation of simple mechanical forces. Rather are the blastomeres the expression of the active intrinsic forces, which control development from the earliest stages unto the end. Gravity, surface tension, cohesion and pressure no doubt are effective, but not to the extent that they become the controlling or coördinating agents in development. The early cleavages are as important as those occurring in later life, and may even be considered more so. "Also the long continued resemblances which exist in the development of these different forms from the earliest segmentation of the eggs are as fundamental and deep seated as are the homologies existing in the adults."

The number of these resemblances in the annelids and molluscs is surprisingly great. In all forms accurately studied, the first three generations of ectomeres give rise to the entire ectoderm. The mesoblast arises at the fourth division of the posterior macromere D. The remaining members of this quartette and the macromeres produce the entoderm. The division and position of the cells up to the twenty-four or thirty-cell stage are identical in many different species. Beyond this point Wilson believes a divergence between the two classes ensues, and that development proceeds upon two entirely different lines. However, subsequent investigators have shown that the supposed differences are more superficial, and that the points of resemblances become more numerous and extend throughout longer periods of development. Lillie ('95) showed that points of resemblance existed in the lamellibranchs and the annelids, and that in both classes there is an essential similarity between the development of the 'first somatoblast.' In annelids this structure develops to a greater extent than in *Unio*, but the two have many points in common.

Mead ('97) and Conklin ('97) showed that the rosette series had the same origin and position in annelids and molluscs, and that in both it probably gave rise to the apical sense organ. According to Conklin, it also gave rise to the cerebral ganglia, while Mead considered this particular point doubtful. Furthermore, Mead in his annelid studies demonstrated that the same cells in five different annelids gave rise to the entoderm; that

the head kidney in *Amphitrite* and *Nereis* developed from the same cells. Conklin further states that the axial relation of all the blastomeres, with the possible exception of the macromeres, are the same in both the annelids and molluscs, and that the larval mesoblast in *Crepidula* and *Unio* arises from the same group of ectodermal cells.

Heath ('99) found that the prototroch in annelids and molluscs was homologous, and that the twenty-two to twenty-five cells concerned have exactly the same origin, direction of cleavage, and destiny. Also that the remainder of the first quartette, forming the head vesicle with its rosette series and molluscan cross cells or intermediate girdle cells, has in all probability, the same fate in both. He found many other resemblances and concludes:

Thus it is seen that not only in the origin and position of the various quartettes do resemblances appear, but that the early cleavage of these are in many cases cell for cell the same. In later stages close cell homologies cease, but the relation of the cell groups and their development in giving rise to larval or adult structures follow along much the same path. After passing these facts in review and considering the various structures in detail and modifications which they undergo, one fact presents itself with greatest clearness, that between *Ischnochiton* and the annelids the resemblances are more fundamental and closer than are the differences.

For a more direct comparative study of *Bdellodrilus* with the annelids and molluscs, special references will be made to *Clepsine* (Hirudinea), *Dinophilus* (Polychaete), and *Unio* (Lamellibranch). In all these forms the first and second cleavages are meridional and divide the eggs into four unequal macromeres (text figs. 19-22). In *Dinophilus* C and D are approximately posterior and A and B are anterior. In the other three forms B is anterior, D posterior, C right and A left. In each case D is the largest cell; A, B and C are nearly equal; B is usually the smallest when variation occurs. The eight-cell stage has the same structure, and in all probability arises in the same manner in the four forms, the only apparent difference being the much greater relative size of the ectomeres in *Dinophilus* than in the three remaining forms. The first cleavage plane in *Bdellodrilus*

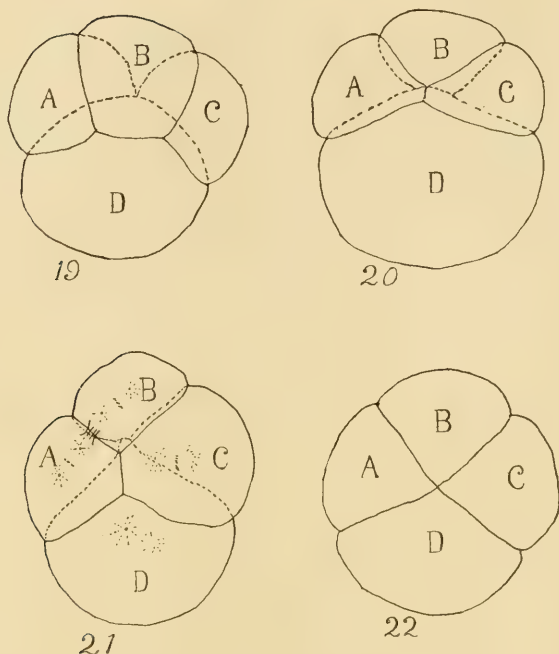


Fig. 19 Four-cell stage of *Unio*, upper pole (after Lillie).

Fig. 20 Four-cell stage of *Bdellodrilus*, upper pole.

Fig. 21 Four-cell stage of *Dinophilus*, upper pole (after Nelson).

Fig. 22 Four-cell stage of *Clepsine*, upper pole (after Whitman).

occurs at nearly right angles, while in *Unio* and *Clepsine* it is inclined at an angle of about forty-five degrees to the sagittal plane of the future adult. In *Dinophilus* the direction of the first cleavage is in doubt. The second cleavage plane in *Unio*, *Clepsine* and *Bdellodrilus* occurs at an angle of about forty-five degrees to the sagittal axis. The origin of the ectoderm, the entoderm and the mesoderm is approximately the same in each form.

1. THE FIRST SOMATOBLAST

The first somatoblast in each instance is derived from the large posterior macromere D (text figs. 23-26). The cell d^2 (X) is extremely large and occupies a median posterior position. In *Clepsine* (Whitman) d^2 (X) is called the 'neuro-nephroblast.'

It divides into two, four and finally eight large cells called the teloblasts; the middle stratum of the germ bands is derived from them. These eight teloblasts are arranged into two groups of four cells each. Each group, which later is composed of four rows of cells, produces the middle stratum of the germ band on the corresponding side. The inner row of each band lies ultimately near the median ventral plane and gives rise to the corresponding half of the nervous system. The adjoining rows—'nephroblasts'—give rise to the nephridia. The derivatives of the outer row are still in doubt, but probably take part in the formation of the ectoderm.

In *Dinophilus* (Nelson) d^2 (X) is formed by a laeotropic division of the macromere D (text fig. 25); D is much smaller than X. Immediately after the formation of X, x^1 is budded off to the right at a low level. Next x^2 is budded off to the left at a higher level than x^1 ; x^3 is next formed by a dexiotropic division from the dorsal side, a little to the left. Next X divides equally and produces X and X, right and left. These two large cells correspond to the proteloblasts in *Bdellodrilus*. Finally X on either side divides equally, and produces the two teloblasts on each side of the median plane. These four cells, according to Nelson, correspond to the posterior teloblasts of *Nereis*. They also correspond to the neuroblasts and nephroblasts of *Bdellodrilus*. The division of X in *Dinophilus* and *Nereis* differs no more than do the corresponding divisions in *Nereis* and other annelids (*Amphitrite*, etc.). At the time of the closure of the blastopore in *Dinophilus*, the descendants of X are distributed dorsally and laterally to the posterior stem cells. In *Neries* the main bulk of the descendants of X lay on the vegetal side of the stem cells.

In *Unio* (Lillie) the 'first somatoblast' X is formed by an unequal division of D (text fig. 24) in a median posterior position; x^1 is budded off from X, just behind C on the vegetal pole; next x^2 is budded off from X symmetrically with x^1 on the right side, just posterior to d^3 ; next x^3 is formed from X towards the apical pole, posterior to $d^{1,2}$; then x^4 is budded off from X anteriorly, towards the vegetal pole. This division of X does

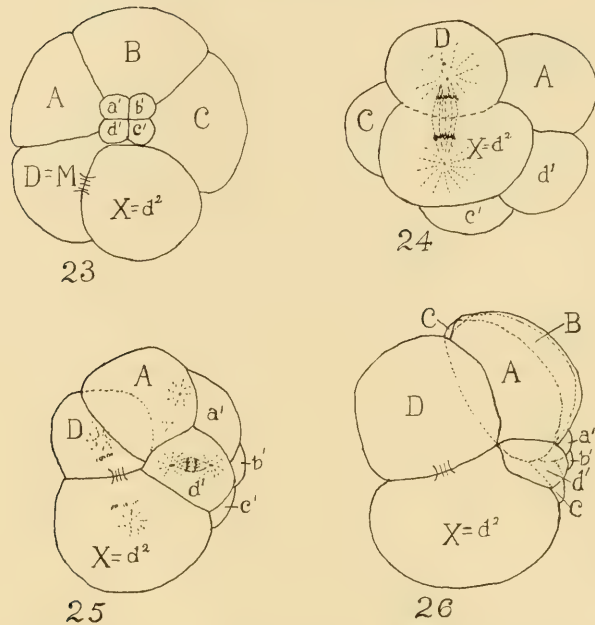


Fig. 23 Nine-cell stage of Clepsine, upper pole (after Whitman).

Fig. 24 Nine-cell stage of Unio from behind (after Lillie).

Fig. 25 Nine-cell stage of Dinophilus, left side (after Nelson).

Fig. 26 Nine-cell stage of Bdellodrilus, left side.

not occur in this manner in *Dinophilus*, *Bdellodrilus* or even in *Nereis*. The fourth division in the above three forms is equal and bilateral, while in *Unio* the fifth cleavage of X is the first bilateral division and forms X, X right and left. Next X, X on either side divides nearly equally and gives rise to the shell gland. These four cells might be regarded as the posterior teloblasts, which occur in other forms, as in *Nereis* and *Dinophilus*.

In *Bdellodrilus* X is formed by an equal division of the macromere D (text fig. 26), and takes a median posterior position. First x^1 is budded off from X to the right, posterior to C. Then x^2 is budded off to the left, symmetrical with x^1 and posterior to d^3 . Next x^3 is formed from the median dorsal anterior edge of X, between d^1 and c^1 . Now the first bilateral division of X takes place and forms the proteloblasts X, X, right and left. Each of the proteloblasts bud off x^4 , x^5 and x^6 respectively. At the

next division each proteloblast divides nearly equally, and gives rise to $X^{(1)}$, neuroblast, and $X^{(2)}$, nephroblast, on each side of the median axis of the embryo. Next each nephroblast divides nearly equally and produces $X^{(2)}$ and $X^{(3)}$. Now a very interesting thing happens; either $X^{(2)}$ or $X^{(3)}$ divides (but never both in the same egg) and produces the three nephroblasts on each side, which are designated as $X^{(2)}$, $X^{(3)}$ and $X^{(4)}$. In Clepsine only two of these teloblasts are concerned in the formation of the nephridia. The lateral teloblasts, as stated above, are probably ectodermal.

These four forms unquestionably show that there is a marked similarity in the cleavage of the 'first somatoblast,' not only in widely different individuals in the same group, but in individuals of widely separated groups. This comparison could be extended to other groups or forms, but the above will suffice for our purpose.

2. THE SECOND SOMATOBLAST

In Clepsine, the 'second somatoblast' has rather a unique origin. It is formed at about the twelve-cell stage. D, after the formation of X, becomes directly the 'second somatoblast' or M. (These cells are differently designated by Whitman; D is represented by x, X by x^1 and the mesoblasts by x and xy.) M divides nearly equally and produces the right and left mesoblasts, from which the inner stratum of the germ bands is formed.

In Bdello-drilus, M is formed by a very unequal division of the macromere D, at the twenty-four-cell stage (fig. 85). The larger cell or M is formed in front of X. It is inclined a little to the left of the median axis. The first division of M is equal, producing the mesoblasts, one on either side. These primary mesoblasts now bud off a number of small cells, directly beneath d^1 and c^1 (figs. 86-87). It is very difficult to make out the exact number of these small cells, since they are not visible externally. There are at least twelve formed, six on either side from each mesoblast. After this small group of secondary mesodermal cells are formed, the mesoblasts M, M, give rise to

PLATE 7

EXPLANATION OF FIGURES

- 78 Two-cell stage, horizontal section; CD dividing.
- 79 Four-cell stage; horizontal section taken above the center of the egg.
- 80 Same as the preceding, with plane of section below center.
- 81 Nine-cell stage, parasagittal section, to right of median plane.
- 82 Horizontal section of a nine-cell stage, taken four sections from the top.
Taken from embryo composed of 21 sections, each eight micra in thickness.
- 83 Same as the preceding; sixth section from top.
- 84 Same as figures 82 and 83; fifteenth section from top.
- 85 Parasagittal section of a twenty-four-cell stage; plane of section little to left of median axis. This figure shows the unequal division of the macromere D in the formation of the second somatoblast.
- 86 Thirty-three-cell stage. Parasagittal section to left of the median plane. Shows the formation of the secondary mesodermal cells (m cells) from the primary mesoblasts.
- 87 About the same stages as the preceding, to show the distribution of the yolk in different cells.
- 88 Horizontal section of an eighteen-cell stage, fourth section from top. Series composed of 20 sections, each eight micra in thickness.
- 89 Same as the preceding; seventh section from top.
- 90 Taken from series same as figure 88; eighth section from top.

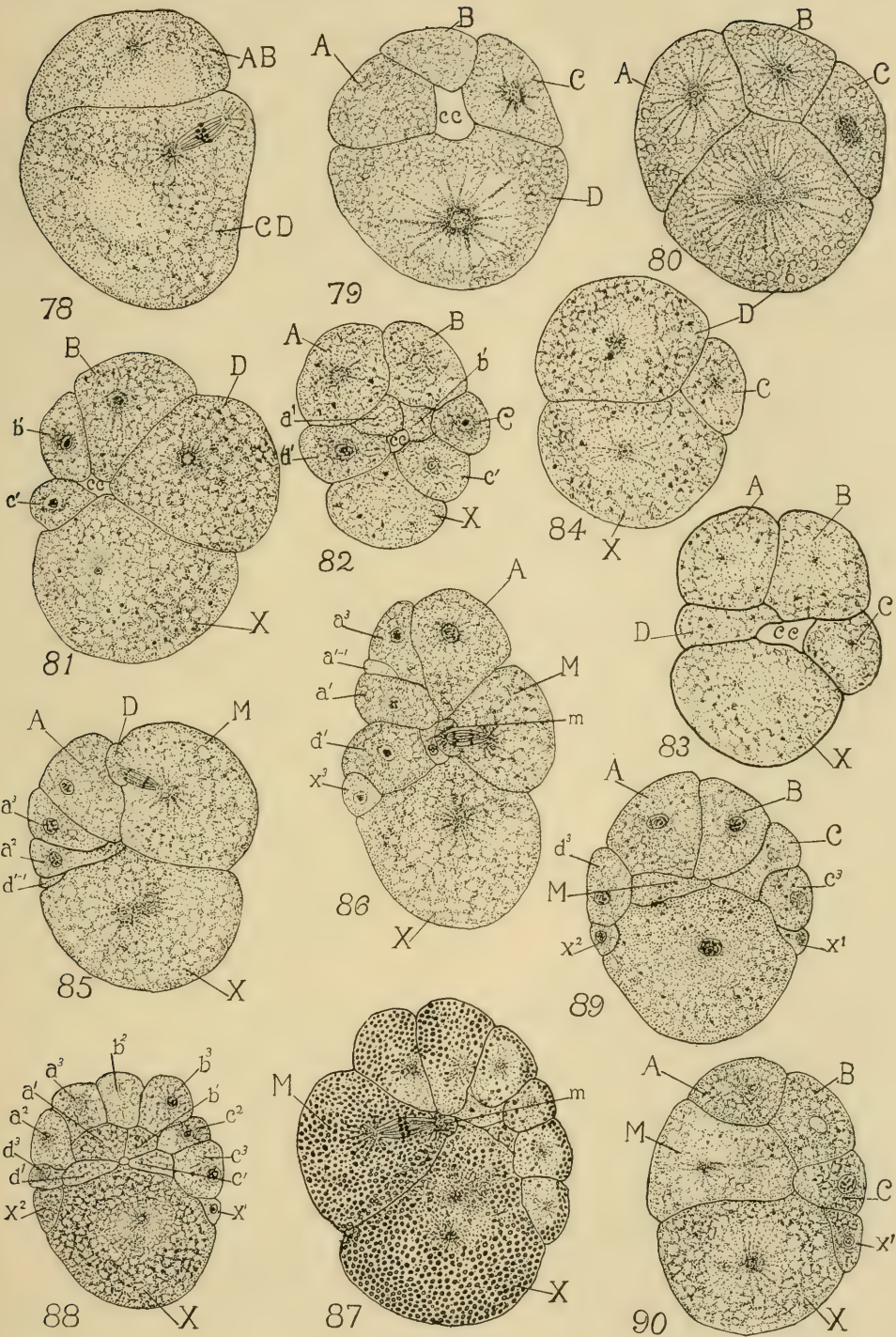
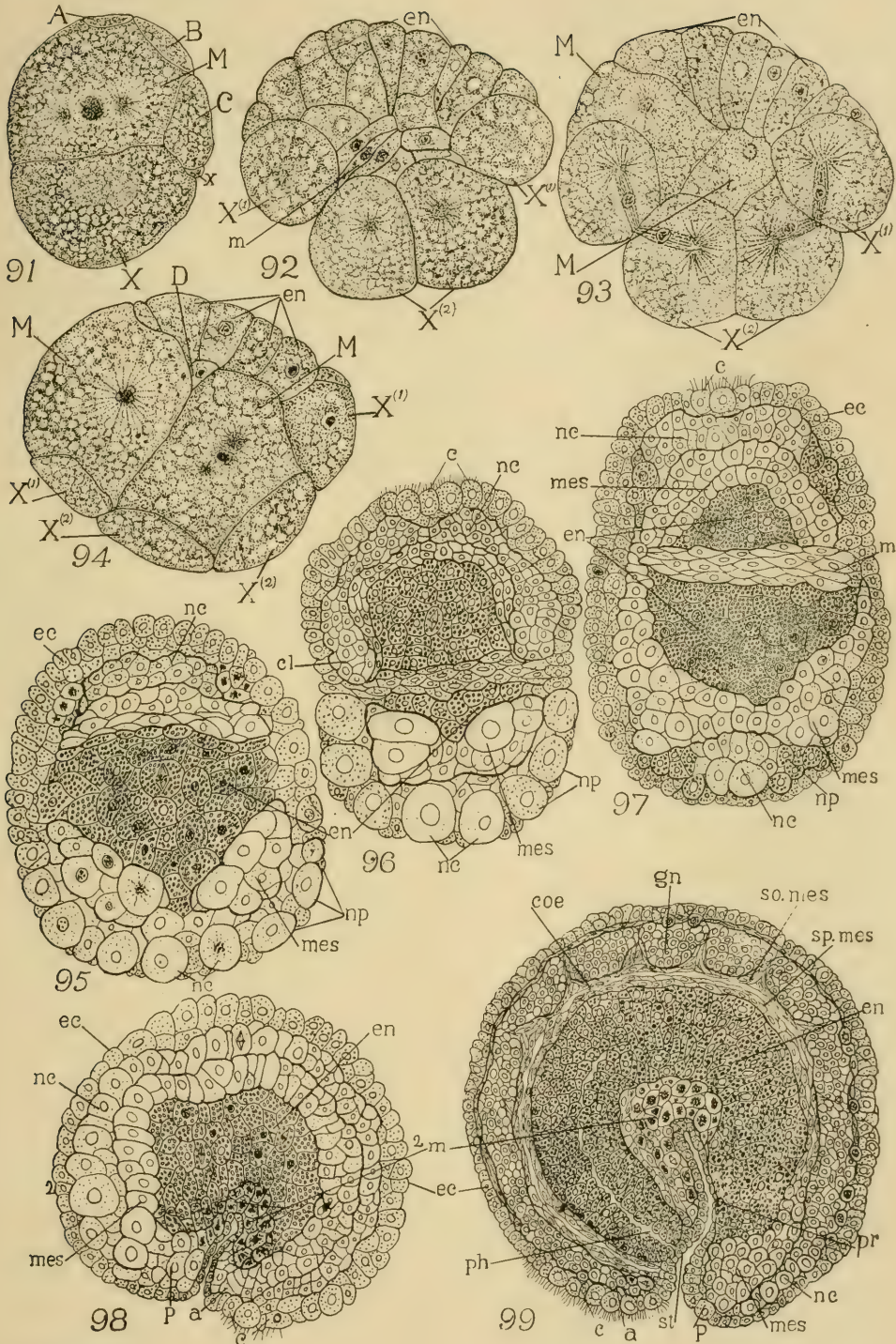


PLATE 8

EXPLANATION OF FIGURES

- 91 Taken from the same series as figures 88 to 90; eighth section from the top.
- 92 Horizontal section of an eighteen-cell stage; sixth section from the top; shows the neuroblasts and nephroblasts.
- 93 Same as the preceding; tenth section from top; this figure shows the persistence of the cleavage spindles after the cell membranes are distinct.
- 94 Same as 92 and 93; fourteenth section from top; taken from a series of 20 section each eight micra thick. This figure shows the upper side of the macromere D wedged in between the other cells.
- 95 Transverse section of an embryo represented by figure 64; section taken at plane 2 — 2, or at a region corresponding to plane 2 — 2, of figure 98.
- 96 Transverse section of stage corresponding to figure 63; section taken at plane 2 — 2; figures 95 and 96 shows germ bands only partially covered by the ectoderm.
- 97 Transverse section of embryo represented in figure 69; section taken at level marked by line 2 — 2; here the germ bands are completely covered by the ectoderm.
- 98 Longitudinal section, near median line, of stage represented by figure 69.
- 99 Longitudinal section of an embryo represented by figure 71; plane of section near median line.



the inner stratum of the germ bands. The plane of division in the formation of the primary mesoblast is at right angles to that of the secondary.

In *Dinophilus*, M is formed at the twenty-nine-cell stage, by an unequal division of D. M is much larger than D, as in *Bdellodrilus*, and is in front and below X, slightly to the left of the median plane. The division of M is now delayed until the seventy-two-cell stage, when two small cells are budded off anteriorly towards the vegetal pole, close to the line of junction of the two mesoblasts. At the next division two small cells are budded off, one on either side of the first pair. The following cleavages are teloblastic and produce the mesoblastic bands. The mesoblasts do not move into the cleavage cavity as in many other annelids, but remain on the surface until covered by the ectoderm.

In *Unio*, at the thirty-two-cell stage, M is formed by a very unequal division of the macromere D. The first division of M is equal and bilateral. Their position is immediately behind the entomeres. The next division of the two mesoblasts is very unequal, two small cells, m, m, are budded off at the posterior lip of the blastopore. Later the mesoblasts are included within the segmentation cavity, where they take up their final position behind the archenteron and give rise to the definitive mesoblastic germ bands with lateral teloblasts.

From the forms compared above it is very evident that there is a remarkable similarity with *Bdellodrilus*, not only in the early cleavage stages, but in the establishment of the germ bands as well. Thus cells having the same origin and lineage have the same final result in a wide series of forms (d^4 the mesoblasts). Again, cells of unlike origin have a different fate (first and second somatoblasts); or cells of a different origin may have the same fate (d^4 of annelids and the second and third generation of ectomeres in polyclades). Then cells of the same origin may have a different fate ($a^{2.2}$ in *Unio* and *Bdellodrilus*). These contradictions, however, are far less striking than the resemblances. The 'first somatoblast' in each of the above four forms gives rise to the ventral plate and all or nearly all of the trunk ectoderm, while

the 'second somatoblast' produces the definitive mesodermal elements of the adult animal.

3. VARIATIONS IN THE METHOD OF MESODERMAL FORMATION

All annelids and molluscs which have been carefully studied show that the ectoderm arises from the three generations of ectomeres, the mesoderm from M and the entoderm from the remaining cells. There are, however, a few minor variations in forms like *Clepsine*, *Crepidula* and *Nereis*. In polyclades the mesoderm is directly associated with the ectomeres. The second and third generations of ectomeres, as in *Discocoelis*, produce the mesoblast, and the macromeres the entoblast. In molluscs and annelids the mesoderm is more closely associated with the macromere D. There is considerable variation in the cleavage of D in the formation of the 'second somatoblast;' in some forms D is given over entirely to the mesoderm; in other forms it shares equally, or in still others it contributes but little to the mesoderm.

Forms in which the mesoblast has two sources

(a) *Ecto-mesoblast* and (b) *coelo-mesoblast*. In *Thalassema*, Torrey distinguished between ecto-mesoblast, from the ectoderm and coelo-mesoblast from M. He states that the coelomesoblast is present in two bands, each consisting of five sub-equal cells. These are closely applied to the body wall and lie in the usual position on each side of the neural rows, but are more widely separated than in most annelids. The ecto-mesoblast on the other hand, derived from the first and second quartette of ectomeres, is present in great abundance and many of the cells have already undergone considerable differentiation in the formation of the muscles. He further states that the two mesoblast cells, M, M, are the last to sink in at gastrulation, instead of the first, as in the case where development is more direct (*Nereis* and *Amphitrite*). The two coelo-mesoblast bands of five cells each are shown to have the same origin and early history as the mesoblast bands in other annelids. The coelo-mesoblast, which is meagerly developed in the trochophore, is clearly correlated with

the long duration of its free swimming and almost stationary larval existence. In all forms where there is a trophophore stage of long duration, as is the case in all annelids with equal cleavage, the two coelo-mesoblast cells do not, in the early stages at least, bud like teloblasts. This is true in Hydroides, some species of Polygordius, in Thalassema and Podarke.

Many of the annelids and molluscs show that the so-called ecto-mesoblast (designated as larval mesoblast or as mesenchyme by some authors) arises from certain ectodermal cleavage cells of the second or third quartette of ectomeres and is entirely independent of the coelo-mesoblast. In Thalassema (Torrey) ten large ectodermal cells sink in from the ectoderm and give rise to all the mesenchyme. Three of these cells are from the a, c and d quadrants of the third quartette and seven from the first quartette of ectomeres. The most important source of mesenchyme in Thalassema is from the three cells of the third quartette ($3d^{2.2.2.1}$, $3c^{2.1.2.1}$, and $3a^{2.2.2}$). The first two sink into the cleavage cavity, just before gastrulation and at first lie close to the two coelo-mesoblast cells. They soon migrate laterally and bud off simultaneously small cells toward the M cells. They divide like teloblasts, but in the reverse order to the ordinary direction. So close is the connection of these cells with the coelo-mesoblast that one would be certainly led to think that they formed a part of these bands, unless their cytogeny had been carefully followed. Similar conditions are described by Treadwell in Podarke obscura. The progeny of these two cells form almost the entire mesenchyme of the post throchal region and become differentiated for the most part into muscles of the digestive tract. The progeny of the other ectodermal cell migrates to the mid-ventral line. The ecto-mesoblast cells of the first quartette sink into the primary body cavity later than those of the third; their exact cell lineage has not been traced, but probably give rise to gut musculature. This mesoblast has commonly been considered as purely larval and transitory. In some instances it is possible to determine its exact origin, but in many others merely the general region from which it arises.

During the last thirty years embryologists have differed in their conception of the origin of the mesoderm and of its phylogenetic significance. Hatschek ('78) was among the first to distinguish between mesenchyme and mesoderm, but held, after studying the embryology of *Polygordius*, *Echiurus* and *Eupomatus*, that these two morphologically different mesoblasts, arise from a common foundation. This same view was later put forth by the Hertwigs ('81) in their 'Coelomtheorie,' which, according to Meyer, has formed the foundation of all later work on mesoderm. Roule ('89 and '94), Burger ('91 and '94), Fraipont ('88), Häcker ('95), and others, have described the mesoblast as having a single origin. On the other hand, those who have studied the embryology of annelids and molluscs, consider the origin of the mesenchyme distinct from that of the mesoblast or coelo-mesoblast. This later view was first described by Kleinenberg ('78 and '86), and later by Whitman ('87), by Berg ('90), by Schimkewitsch ('94), by Meyer ('01), by Torrey ('03), and others.

A larval mesoblast was first described by Lillie ('95) in *Unio*. It arises asymmetrically from the derivatives of $a^{2,2}$ and later migrates into the segmentation cavity, where it divides equally and becomes symmetrically arranged on either side of the mid-line. The derivatives of these two cells become metamorphosed into 'myocytes' and larval adductor muscles, which are functional only during larval life.

Treadwell ('97) regards both mesenchyme and mesoderm as morphologically the same tissue, the apparent difference in their mode of origin being of no significance. And, further, Wilson regards the larval mesoblast (ecto-mesoblast, because of its origin from the ectoderm) as a distinct tissue from that of the definitive mesoblast or ento-mesoblast, and states that it is homologous with the mesenchyme of the turbellarian ancestors of the annelids, while the mesoblast from which the adult structures arise is phylogenetically younger and is represented prophetically in the ontogeny of such a form as *Discocoelis* (polyclade) by the peculiar lateral division of M, and states that the ecto-mesoblast and endo-mesoblast are phylogenetically of

different origin. This same point was previously urged by Meyer.

The condition, however, found by Wilson in *Nereis* and *Lumbricus* does not indicate a hard and fast distinction between the two kinds of mesoblast. In *Nereis*, cells from the anterior end of the germ bands separate early and pass forward into the segmentation cavity where they give rise to the larval musculature. This corresponds exactly in structure and function with the larval mesoblast of *Unio* (Lillie) and *Podarke* (Treadwell). In *Lumbricus* the origin of the mesenchyme is similar to that in *Nereis*. These two kinds of larval mesenchyme have also been described by Eisig ('98) as occurring in the same individual (*Capitella*, a polychaete annelid).

In *Thalassema* and *Podarke* the larval mesenchyme arises directly from the ectoderm, while in *Nereis* and *Lumbricus* it arises from the anterior ends of the mesoblast bands. According to Treadwell, no one has yet proven that no 'mesenchyme' arises from the germ bands in cases where a larval mesenchyme exists. If we accept Wilson's view that mesenchyme and mesoderm are different phylogenetically, we must regard the two sets of larval mesenchyme which have the same structure and function, as non-homologous, or we must regard the mesenchyme and mesoderm as morphologically the same tissue and the difference in their modes of origin as of no significance. Furthermore, Wilson has pointed out that the trochophore, as it occurs at present, is more than a mere ancestral stage, for it contains in a concentrated form the anlage of the whole future body. According to Mead, the ectoderm behind the first septum in *Amphitrite* arises from a group of cells which surround the procotodaeum of the young trochophore and are descended from a single cell, the 'first somatoblast.' The same is true of other trochophore forms. There is no need to assume phylogenetically a new formation of ectoderm for the body as distinct from that of the head. Neither is there any necessity to assume a distinct phylogenetic origin of the larval mesoblast from that of the mesoderm.

It is evident that in *Nereis* and *Lumbricus*, both kinds of mesoblast have the same origin, and simply shows a more complete concentration of the mesoderm than in *Thalassema* and *Podarke*, where the mesenchyme is formed direct from the ectoblast. The mesoblast cells collected at the posterior end of the trochophore, which are derived from M, represent the mesoderm of the body. It is morphologically continuous with that of the head, as in *Nereis*, and is concentrated at this point to provide for the elongation as new segments are formed. The difference in the concentration of the mesodermal elements, as to whether they have a single or double origin in no way interferes, as already pointed out, with the morphological unity of the tissue, and as to the source of its origin, whether from the ectoderm or from the endoderm phylogenetically, we are not able to say (Treadwell).

Meyer ('01) in his study of the phylogenetic significance of the two kinds of mesoblast, gave a view directly opposed to that expressed by Treadwell. After an exhaustive review of the whole mesodermal question, he concludes that the great mass of evidence, both embryological and anatomical, points to the conclusion that in annelids, at least, there are two entirely distinct forms of mesoblast, the ecto-mesoblast (primary mesoblast) and the coelo-mesoblast (secondary mesoblast). Of these two he considers the primary mesoblast to be phylogenetically the older, and as a rule, to be derived from the ectoderm. The coelo-mesoblast, on the other hand, is regarded as a later formation, which has originated from the gonad cells.

The formation of the ecto-mesoblast in annelids and molluscs from certain cells of the first, second, third and fourth generation of micromeres, can well be regarded as vestiges or survivals of the process which occurs in all four cells of the second and third quartettes of certain polyclads. The origin of the ecto-mesoblast from the ectoderm in annelids and molluscs, partially bridges the gap between them and the polyclads. In order to have a complete homology of the mesoderm in the polyclads, annelids and molluscs, it is necessary to find a polyclad in which there is a double origin of the mesoderm. The development of the polyclad *Leptoplana* (Wilson) is the nearest representative to complete the homology. In *Leptoplana* only a part of the four quadrants of the second quartette contributes to the entire mesoderm, the typical condition in polyclads being that all of

the second and third quartette is mesodermal. The behavior of d^4 in the polyclad *Discocoelis*, is also very suggestive. Here the division of d^4 is equal and gives rise to two symmetrically placed cells at the posterior end of the embryo, comparable to the primary mesoblasts found in annelids and molluscs. Some investigators have even suggested that these two posterior cells in the polyclads may give rise to the mesoblast bands in this particular group. This latter point, however, has never been verified.

Table 3 shows that the first, second, third and fourth generation of micromeres, in a series of widely separated forms, may contribute to the formation of the mesoderm.

TABLE 3

	1ST GEN.	2D GEN.	3D GEN.	4TH GEN.
Annelids:				
Thalassema	part of a, b and c quad's	none	1 cell each of a, b and c quadrants	d^4 part mes.
Bdellodrilus	none	none	none	d^4 all mes.
Molluscs:				
Unio	none	$a^{2.2}$ (larval)	none	d^4 all mes.
Crepidula	none	a^2, b^2, c^2	none	d^4 part mes.
Physa	none	none	b^3, c^3	d^4 part mes.
Podarke	none	none	$a^{3.2.2.2}$ $c^{3.2.1.2}$ $d^{3.2.2.2}$	d^4 part mes.
Polyclads:				
Discocoelis	none	all mes.	all mes.	none
Leptoplana	none	part of each quadrant	none	none

In case of the ecto-mesoblast a complete series could be arranged, in which all of the cells of certain quartettes contribute to the mesoblast, to those forms in which only a small part of certain quartettes is mesoblastic. Again in case of the coelo-mesoblast we have a wide range of variation, in which all of d^4 is mesodermal, to those in which only a small part of d^4 is mesodermal. As far as records show, *Capitella* is the only annelid in which

d^4 does not contribute to the coelo-mesoblast. Here then we have quite a unique series ranging from those forms where the entire mesoderm is ectodermal in origin, or where it is both ectodermal and entodermal, to those where it is entirely entodermal. From the above it is evident that the entire mesoblast of polyclads is derived from the ectomeres, and, if homologies be any significance, it would be fair to conclude that this mesoblast is represented by the ecto-mesoblast in the annelids and the molluscs.

The origin and development of the mesoblast in *Bdellodrilus* contributes but little to the phylogenetic significance of the primary and secondary mesoblast. Here, beyond question, when considered from the standpoint of their origin, they are one and the same tissue. Both are formed directly from the primary mesoblasts. The secondary mesoblast cells are budded off from the two primary mesoblasts before the germ bands begin their development. Similar conditions are found in other forms, as in *Lumbricus*; here, however, the secondary mesoblast is formed later directly from the anterior ends of the mesoblastic germ bands. The difference is only in the point of time in their formation. In *Bdellodrilus* there can be no hard and fast distinction made between the two kinds of mesoblast. Both must be considered as the same tissue phylogenetically.

4. VARIATIONS IN THE SOURCE OF THE ENTODERM

In general, as stated above, the ectoderm originates from the three generations of ectomeres, the mesoderm from d^4 , and the entoderm from the remaining cells. The origin of the three germ layers, however, depart somewhat from the above rule in some of the annelids and molluscs. In some species cells from the first, second and third quartettes contribute to the mesoderm; in others d^4 gives rise to entoderm as well as mesoderm. In all annelids and molluscs, A, B and C, after the formation of the first three sets of ectomeres, are distinctly entodermal. The macromere D, after the formation of d^4 , is likewise entodermal. In some forms D is the same size as its fellows, in others

it is reduced until it is little more than a mere nucleus, while in others it has completely disappeared as an entomere, and is given over entirely to the formation of mesoderm.

In annelids, in a gradually decreasing series, D (*Nereis*) is the same size as the entoblast cells A, B and C. In *Dinophilus* it is about half the size of these cells. In *Bdellodrilus* D is little more than a mere nucleus; while in *Clepsine* D is given over entirely to the formation of the mesoderm. In molluscs it is a fairly common condition to find the entoblast cell D smaller than A, B and C, or even greatly reduced. In *Crepidula* it is very little reduced; in *Unio* it is more than half reduced, while in *Ischnochiton*, D is often little more than a mere nucleus. The second somatoblast, M, may contribute to the formation of entoderm as well as mesoderm. In forms like *Crepidula* M is mostly entodermal. In *Fiona* (Casteel) the division of M in the formation of the entoderm is very similar to that in *Crepidula*. In *Unio* two small cells are budded off from M, which lie near the entoderm, and are probably concerned in the formation of that layer.

In some of the annelids the primary mesoblasts bud off small cells directly posterior to the macromeres. This number varies; in *Nereis* there are six to ten, and in *Aricia* there are but two. In many of the other annelids and also in some of the molluscs, where their cell lineage has been traced, it is found that these small cells give rise to entoderm. There are at least sixteen to twenty species of annelids and molluscs in which similar cells have been found (small cells from the primary mesoblasts.) Diverse accounts of their behavior and fate have been given by different investigators. Table 4 shows the fate of these small cells in a few of the annelids and molluscs.

In the mollusc *Alpysia*, according to Carazzi, each primary mesoblast buds off four small cells. Three of these are mesoblastic and one is entoblastic. This interesting condition might be considered as a transitional form or as a connecting link between those forms in which these small cells are entirely mesodermal and those in which they are entodermal. Again we could arrange a series of annelids and molluscs in which at one extreme

TABLE 4

ENTODERM	MESODERM	NOT CERTAIN
Crepidula	Amphitrite	Dreissensia
Nereis	Arenicola	Patella
Podarke	Umbrella	Spio
Thalassema	Planorbis	Serpulorbis
Fiona	Unio?	Cyclas
Ischnochiton	Limax	Aricia
Physa fontanalis		
Physa hyponurum		
Aplysia		

the entoblast derived from M is greater in amount than the mesoderm, as found in *Crepidula*, and at the other extreme, where but two rudimentary cells of M are entoblastic, as in *Aricia*.

According to Wilson, a series of this nature may indicate a gradual elimination of the entodermal element from the macromere D of the fourth quartette, and finally its complete transformation into the mesoblast. Kovalevksy ('71) suggested that this transformation shows quite forcibly that the mesoblast pole cells are to be regarded, phylogenetically, as derivatives of the archenteron, because of their close association with the posterior entoblast cell, D.

The primary entoblasts, A, B, C and D, undergo but little change until late development in those forms which possess a larval stage, and may remain in this condition until after the trochophore is developed, or until after the blastopore is closed. In those individuals with a fetal type of development, they often remain distinct until after the germ bands are completely formed, as in *Clepsine*.

GENERAL ADAPTATION AND INTERPRETATION OF CLEAVAGE

The cleavage of eggs of widely separated forms exhibit unique resemblances. At certain stages of development these resemblances exceed their differences. Is the persistence of these features due to the influence of ancestral inheritance, or are they due more to the adaptive conditions of their environment, to

meet the highest need of the developing animal? It has been demonstrated, again and again, in annelids, molluscs and even in polyclads, that homologous cells of like generations give rise to like parts in the developing embryo and the adult. The occurrence of these conditions in such widely separated forms furnishes a very interesting and important phase in the study of cell lineage. The tendency has been rather to emphasize these resemblances, than to give special stress to the exact conditions which occur in any one species in its different stages of development. It is true, however, that the general form of cleavage may be inherited from a long series of ancestors, probably from some of the Turbellarian worms. But the problem of more direct importance in any one group is, why such variation in the size, form, direction and rate of cleavage?

1. IN THE CLEAVAGE OF BDELLODRILUS

In *Bdellodrilus* we have a determinate type of cleavage, i.e., the fetal as well as the adult structures can be shown to have a definite or direct cell lineage, and can be traced back to the unsegmented egg. The structure of the ovum is quite homogeneous, and at the time of maturation, the egg can be definitely oriented as to the future axis of the body. Before the first cleavage is complete, the parts of the ovum which give rise to the different germ layers can be traced or ascertained with a fair degree of accuracy, i.e., definitely localized parts which give rise to definite organs or structures.

"Adaptation in cleavage can manifest itself only in three possible ways or modes of cleavage variation, which are, as has been pointed out by Lillie, Mead, Conklin and others, the following: first differences in the rate of cleavage; second differences in the size; and third, differences in the direction of cleavage."

The general plan of cleavage in *Bdellodrilus*, is similar to that of other forms. The ectoderm is derived from the four basal cells, by three successive horizontally formed cleavages. The mesoderm from a fourth cleavage of the posterior macromere D and the entoderm from the remaining cells. The first cleavage

in *Bdellodrilus* is meridional and very unequal. In the two-cell stage the larger cell is posterior and the smaller cell anterior. The larger cell divides first and very unequally, while the smaller cell divides nearly equal (text figs. 1-3 and fig. 5). In the four-cell stage D is posterior, C right, A left and B anterior, inclined a little to the right. Thus it is very evident that the four-cell stage illustrates a difference in the rate of cleavage, a difference in the size of the cells, and a difference in the direction of the cleavage. The significance of these variations may be emphasized as follows:

a. Difference in the rate of cleavage of cells

If we compare a thirty-two-cell stage of *Bdellodrilus* with other forms or perhaps, better, with an ideal ovum, in which there is a uniform rate of cleavage in the formation of the cleavage cells, a uniform size and a uniform direction of cleavage, a distinct variation occurs as shown in table 5.

TABLE 5

	CREPIDULA	IDEAL OVUM	NEREIS	BDELLO- DRILUS
First generation of ectomeres.....	12	16	16	8
Second generation of ectomeres.....	9	8	8	11
Third generation of ectomeres.....	5	4	4	4
Mesoblast.....	2	0	0	2
Entoblast.....	4	4	4	7
	—	—	—	—
	32	32	32	32

It is evident that in the first generation of ectomeres *Bdellodrilus* departs very far from the ideal condition. The first generation contains eight cells instead of sixteen. This means that the cells have divided more slowly than in the ideal ovum. In an ideal ovum these cells form the prototroch and the entire region in front of it, with the apical plate in the center. In *Bdellodrilus*, this region is degenerate and no trace of the apical plate appears. This indicates an adaptive modification—eight cells instead of sixteen—due to a degenerate frontal region.

In the second generation of ectomeres, the ideal number is

eight, while in *Bdellodrilus* it is eleven. This increase above the ideal is due entirely to the rapid succeeding divisions of one cell— d^2 , the first somatoblast. The other cells of the quartette have not divided, while d^2 has given rise to three new cells. Does the behavior of d^2 suggest any significance, or is it adaptive? From d^2 the ectoderm of the trunk region, the nephridia and the entire nervous system is derived; d^2 is not only the largest but the most actively dividing cell of the entire embryo; hence its rate of cleavage is well adapted to its resulting formations.

The number of ectomeres in the third generation is the same as in an ideal ovum; d^3 however is often formed before a^2 or b^2 of the second generation. This interesting phenomenon is due to the tendency of the basal cell, D, and its derivatives to divide more rapidly than those of A, B or C. The differences in the rate of cleavage in the first, second and third generation of ectomeres, no doubt possess prospective significance, looking forward to the definitive parts. This may fairly be called adaptation in the rate of cleavage.

In *Bdellodrilus* the more rapidly dividing cells do not form the first functioning parts. The cells of the first quartette are the first to function, in the production of cilia for the movement of the embryo. The variation in the rate of cleavage is not due to the varying conditions of the media, or the dividing ovum would be uniformly affected, as a whole. Nor is it due to the size of the individual cells, as the largest cells divide more rapidly. At the thirty-two-cell stage the ideal ovum contains four entodermal cells, while in *Bdellodrilus* there are seven. Here the cleavage is carried to the end without any resting stage of the four basal cells. This is due to the fact that the larva develops very rapidly and the entodermal cells must keep pace with the rapid development in order to reach their final position, just where they are needed.

b. Variation in the size of cells

The relative sizes of the cells in the early cleavage of the eggs of *Bdellodrilus* are adapted to the later developing parts.

The largest cell at the four-cell stage is D. Its first division is very unequal, and the smaller cell is less than one-tenth the size of the larger. It is the first ectomere of the first generation formed. The second division, in most instances, is equal; when unequal, the largest cell passes into the upper product, and forms d^2 , the first somatoblast. The third division is unequal, and d^3 , the smaller product, is again uppermost; and finally, the fourth division is very unequal and only a small portion remains as the macromere D. The greater bulk, d^4 , becomes the second somatoblast. In each of the above instances the larger cells form a large part of the embryo and the adult, while the smaller cells, in every instance, form a very insignificant portion. The unequal division in each instance is evidently adaptive, for the great bulk of the material passes into the two somatoblasts and gives rise to the muscular, nervous and excretory systems.

c. Variation in the direction of cleavage

Here only some of the special cleavages will be emphasized. The first division of the second somatoblast is equal, and each part forms equal parts of the mesoderm. Next, each primary mesoblast buds off five or six small cells beneath the first quartette of ectomeres. These small cells remain quiescent for a considerable period and later give rise to the dorsal mesoderm. Immediately after these small cells are formed, the mesoblast bands are begun by a forward proliferation of cells from the anterior face of the primary mesoblasts. The plane of division is at right angles to that of the small cells. These bands extend forward between the ectoderm and the entoderm, and at the same time the entodermal cells extend posteriorly between the mesoblast bands and the group of small cells, thus separating the primary and secondary mesoderm. Here the direction of the cleavages place the cells where they are later used in the formation of some special part, adapted for that particular region.

The first somatoblast buds off x^1 to the right, x^2 to the left, and x^3 median dorsal anterior. X now divides equally and

each proteloblast buds off a small cell, x^4 , one to the right of x^1 and the other to the left of x^2 . Again each proteloblast buds off a small cell, x^5 , on either side of x^3 . At the next division each buds off a small cell, x^6 , on the ventral anterior edge. Later, x^7 is budded off from each neuroblast on the ventral side. These small cells give rise to the trunk ectoderm and the larger cells to the nephridia and nervous system. Here again the cells are formed just where they are needed; the smaller on the exterior or outer surface while the larger remain within.

2. ADAPTATION IN THE CLEAVAGE OF OTHER FORMS

In following the variation of cleavage cells in annelids and molluscs, special cells can be arranged in a complete series, from those of an almost insignificant size to an extremely large cell. In following these variations, step by step, we can not fail to be convinced that these variations are adaptive to the future needs and habits of the larva and of the adult animal.

In forms with equal cleavage, the first somatoblast gives rise to the ectoderm of the trunk region. In *Polydorus*, *Podarke*, *Hydroides*, *Eupomatus* and others with equal cleavage, d^2 is the same in size as the cells of the other quadrants. Equal cleavage has been offered by some as due to a lack of differentiation in the early stages but in such forms as *Podarke* with equally cleavage, very early differentiation occurs, and the prominence of these early functioning parts varies according to the size of the initial cell from which they are formed.

In forms with unequal cleavage, the first somatoblast differs in size from the remaining members of the same quartette. Beginning with *Amphitrite*, the relative size of d^2 increases successively in *Chaetopterus*, *Arenicola*, *Nereis*, *Capitella*, *Aricia*, *Spio*, *Clepsine* and *Bdellodrilus*. Those forms with equal cleavage pass through a distinct trochophore stage and are characterized by an almost equatorial prototroch, a very large exumbrella, and with a very slow trunk development. In those with unequal cleavage, especially in the second generation of ectomeres, there is a gradual decrease in the prominence of the

trochophore to its approximate or complete disappearance; on the other hand, there is a gradual acceleration in the time of the trunk development, varying according to the increase in the relative size of the first somatoblast or X. Treadwell states that the extra amount of material stored in the macromere D is in some way related to the amount of somatic and mesoblastic material needed in the future organism. This statement is true of the condition that occurs in such annelids as *Bdellodrilus* and *Clepsine*.

GENERAL SUMMARY

The undivided egg of *Bdellodrilus philadelphicus* is nearly oval. Its median longitudinal axis through the region of the polar bodies corresponds to the median axis of the future adult. The polar bodies occupy the region which later becomes the anterior end of the embryo.

The first cleavage plane is nearly at right angles to the median axis of the resulting individual, and divides the egg into two very unequal parts. The second cleavage occurs at an angle of about forty-five degrees to the first. It divides the smaller cell nearly equally and the larger cell very unequally; the larger cell divides first. In a four-celled embryo the large cell D is posterior, B is anterior, inclined a little to the right; A left and, C right.

The ectoderm is separated from the four macromeres by a series of three oblique cleavages. The first generation of ectomeres is formed in a dextrotropic direction. The second generation laeotropically and the third in a dextrotropic fashion.

In the fourth generation of micromeres, d^4 is mesoblastic. The other cells of the fourth quartette, together with the four macromeres, form the entoderm. The cleavage of the entodermal cells is carried to the end without delay, in the formation of the digestive tract, and the interior of the embryo becomes a solid mass of entodermal cleavage cells, which later become differentiated into the epithelial portion of the alimentary canal. As the core of entodermal cells grows posteriorly,

it separates the primary and secondary mesoderm. The extreme ends of the digestive tract are ectodermal. Among other annelids and also in molluscs, so far as is known, the entodermal cells are not broken up into cells but enter directly into the formation of the digestive tract.

The posterior cell, d^2 (X), of the second generation of ectomeres is the largest cell of the segmenting ovum. The derivatives of X are symmetrically placed with reference to the median plane of the future individual. The large cell, X, gives rise to the trunk ectoderm, the nervous and the excretory systems. The nervous system is derived from the two neuroblasts. The brain is formed from the extreme anterior end of the neural rows.

The largest cell, d^4 (M), of the fourth generation of micromeres, gives rise to the entire mesoderm. It is the first cell to divide in a bilaterally symmetrical manner. The primary mesoblast cells, M, M, bud off five or six small cells each, beneath the first quartette of ectomeres, which give rise to the secondary mesoderm on the dorsal side of the embryo. Immediately after these small cells are budded off, the primary mesoblasts, by a teloblastic proliferation of cells, produce the mesoblast bands.

The embryo increases but little in bulk before the germ bands are formed. The embryo as a whole, during its early stages of development, is extremely plastic and may vary considerably in its transverse and longitudinal axes. The developing embryo is completely turned on itself, and the anterior and posterior ends are in immediate contact. The outer surface is ventral and the turned in portion is dorsal. This peculiarity of development is foreshadowed in the position taken by the early cleavage cells.

At the beginning of the germ-band formation, the embryo begins to rotate on its transverse axis. This movement is due to the action of cilia, which are produced by the ectodermal cells on the median ventro-anterior end of the embryo; the rotation alternates.

As growth continues within the cocoon, the ends of the embryo soon begin to overlap. The embryo may assume almost

any position in the cocoon during its later stages of development. The embryo is completely developed before emergence; the trochophore stage is completely suppressed; the gastrulation is of the epibolic type.

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THE MORPHOLOGY OF NORMAL FERTILIZATION IN PLATYNEREIS MEGALOPS

E. E. JUST

THREE PLATES (THIRTY FIGURES)

1. INTRODUCTION

In a previous paper on *Platynereis megalops*, which described the egg-laying habits, it was stated that insemination takes place in the body cavity of the female and, further, that the eggs will not fertilize when inseminated in sea water. The present paper is a description of the normal fertilization process in *Platynereis*. An experimental analysis of fertilization in *Platynereis* appears elsewhere (Just, '15).

2. NORMAL FERTILIZATION OF PLATYNEREIS

The living egg. The egg of *Platynereis* is compressed and irregular in shape while in the body cavity. Those eggs which happen to be uninseminated when laid gradually round out in sea-water as almost perfect spheres equatorially, but with a rather shorter polar axis. The large, centrally placed, germinal vesicle is slightly elongated in the polar direction. The largest eggs, fully rounded out, measure 180 to 200 μ . They are almost perfectly transparent, have an equatorial ring of oil drops, and a well marked transparent exoplasm or cortical layer of protoplasm with very faint granules forming a delicate mesh. In short, the living egg closely resembles that of *Nereis*; it is larger (but cf. Wilson, '92) not so deeply pigmented, and lacks the characteristic yolk spheres of the *Nereis* egg.

A. Fertilization in the living egg

We may consider the fertilization of the egg under the following heads: (1) insemination, (2) penetration of the sperm, and (3) copulation of the germ nuclei.

1. *Insemination.* In *Platynereis* insemination normally takes place in the body cavity (Just, '14). The eggs, when laid, have the sperm attached within a thin hull of jelly, the secretion of the cortical layer. If the worms be allowed to deposit eggs in India ink ground up in sea-water it can be proved satisfactorily that a hull of jelly, as in *Nereis*, envelops inseminated eggs. This jelly, absent in uninseminated eggs, is formed from the exoplasm of the egg as the result of stimulation through sperm attachment. In sea-water the zone between India ink particles and the vitelline membrane gradually widens, not so much because of the slow diffusion of the jelly from the egg, as because of the swelling of the extruded jelly.

Insemination in some way brings about oviposition. The presence of the sperm in the female is a stimulus to egg laying; as in *Nereis* (see Lillie and Just) the presence of the sperm in the sea-water brings about the shedding of the eggs. The first result of the attachment of the sperm to the egg is jelly formation through cortical secretion, with the consequent formation of the perivitelline space; and this process must begin in the body cavity, since eggs have a thin jelly investment when laid. As in *Nereis* the vitelline membrane is preformed; the sperm does not cause 'membrane formation.'

For twenty to thirty minutes after oviposition, the sperm remains external to the egg. During this time profound changes take place in the egg, many of which doubtless are to be interpreted as changes incident to maturation, the mechanism of which is released with the breakdown of the cortical substance and the consequent formation of the perivitelline space. These changes: breakdown of the germinal vesicle, formation of the spindle, polar body formation, and cytoplasmic movements, are easily followed in the living egg.

2. *Penetration.* In *Nereis* a striking phenomenon of sperm attachment is the fertilization cone (Lillie, '11, '12). In *Platynereis* no sharply defined cone is found. There are, however, cytoplasmic disturbances at the point of sperm entry. In polyspermic eggs the cytoplasm may form a low blunt protrusion, with as many as five spermatozoa attached to it, but this is not

a cone. Twenty-five minutes after oviposition a slender strand of protoplasm may be discerned across the perivitelline space and beneath the point of sperm entry; even this does not seem to be constant, but appears to be formed only in the animal hemisphere. This protoplasmic strand lies, first, in a radius of the egg, but gradually bends so that it now lies almost tangential to the egg. It is found after sperm entry.

After thirty minutes the spermatozoon is engulfed. Often the formation of the sperm aster is discernible, the middle-piece and tail remaining outside. The maturation asters are always visible in the living egg. Mathews ('06) has called attention to the difference between the structure of the asters of living eggs and of fixed material. The difference is certainly striking in *Platynereis*. Instead of the short stiff astral fibres of chrom-osmic material or the long slender ones of mercuric fixation, one sees in the living egg beautiful broad rays sweeping through the cytoplasm.

3. *Copulation of the germ nuclei.* About fifty minutes after egg-laying, the germ nuclei copulate, the cleavage asters form, and at sixty minutes the egg divides unequally. The egg at this time exhibits a stratification of protoplasmic stuffs. During maturation the cytoplasmic currents shift the materials. The equatorially placed oil drops, about eighteen in number, gradually become massed at the vegetative pole, the coarser (yolk) granules lie above these; at the clearer animal pole are the male and female pronuclei. Beneath the polar bodies the cytoplasm is most transparent. The asters are very distinct. One cannot get an adequate picture of these structures from sections. In the living egg they are incomparably clear; large broad rays which bear little resemblance to the short stiff fibres seen in the sections.

The penetration path of the spermatozoon may often be followed, the copulation path always followed. The spermatozoon enters at any point of the egg and through this, as in *Nereis* (Just, '12), the first cleavage plane passes along the copulation path of the germ nuclei.

B. Observations on the sectioned egg

Observations of the phenomena of fertilization in the living egg were supplemented with a study of sectioned material.

Technique. Eggs were fixed in Meves' fluid for thirty minutes, one hour, or twelve hours. Aceto-osmic-bichromate mixtures (Mathews,¹ '99; Bensley); Bouin's fluid, modified by the addition of an equal volume of water; and Gilson's mercuric-nitric mixture were likewise used. Although very destructive to the yolk and oil, the modified Bouin proved helpful in the study of certain details in connection with sperm penetration.

The difficulties of fixation, which are great in this egg, as in *Nereis*, may in large measure be overcome by the subsequent treatment. The following methods were used after fixation with Meves:

(a) Clearing with double distilled anilin oil from 80 per cent alcohol.

(b) Clearing in cedar oil from 95 per cent alcohol.

(c) Clearing in cedar oil from 95 per cent alcohol after treatment with glycerine (eggs put in 70 per cent alcohol plus an equal amount of glycerine).

(d) Clearing in xylol from 95 per cent alcohol or from absolute alcohol.

In all cases xylol was used before imbedding in paraffin or in paraffin with some admixture of Johnston's rubber-asphalt mass. It was found that avoidance of absolute alcohol left the eggs less brittle and therefore less refractory in cutting. By far the most natural contours of both the *Platynereis* and the *Nereis* eggs are preserved through the use of aniline oil after 80 per cent—a clearing agent that I have used successfully for several years. Staining was with iron hematoxylin alone. Sections were cut four micra thick.

Spermatozoa, after fixation, were studied for the most part unstained after the methods of Koltzoff, de Meyer, etc. The iodine mixture recommended by Mayer for *Volvox* proved in-

¹ From the legend of Mathews' figures it appears that he used aceto-osmic-bichromate mixtures.

valuable. For permanent preparations Bensley's staining mixtures were used.

1. *Stages previous to the penetration of the sperm.* The egg of *Platynereis* rivals in structure the beauty of the *Nereis* egg. A section of an uninseminated egg (fig. 1) teased out of the female directly into Meves' fluid gives many of the details. The cytoplasm is sharply marked off into two regions: the exoplasm made up of clear cortex and zone of oil and yolk and the deeply staining endoplasm.

The outer portion of the exoplasm is a mesh of pale blue delicate fibrils, the alveoli of the cortical jelly. The outer limits of this cortical layer—slightly more dense than the deeper portions—is studded with black granules immediately below the vitelline membrane. The inner border of the cortex arises from a zone of closely-packed, deep-staining bodies, from which apparently the walls of the cortical alveoli project. Below this inner border is the region of oil drops which lies in the equatorial zone, among spherules which prove, from their later behavior, to be yolk spheres, although even in the best preparations, the fine granules of which they are composed tend to shrink from their spherical walls (*cf.* Lillie, '11; figure of *Nereis* egg fixed in Fleming). These yolk spheres are evenly crowded against the deeply stained basal area of the cortex. Around the germinal vesicle and closely applied to it is the endoplasmic mass, made up of fine granules which take the stain very tenaciously. Its outer limits are uneven, encroaching on the area of oil drops and yolk spheres as blunt projections.

Scattered throughout the germinal vesicle, as in *Nereis*, are the chromosomes—fourteen tetrads. These lie among many black granules of varying size. Although an attempt has been made to study their number, distribution, etc., and to ascertain any constant characters, nothing now can be said further of them. These granules tend to be spherical and to grade down to minute bodies.

The whole egg, therefore, exhibits a granular structure, both living and fixed, as Mathews some time since ('06) for echinoderm eggs and more recently Kite for some other eggs have shown.

Lillie ('06), too, in a most elaborate study on the egg of *Chaetopterus*, has determined the granular structure of the cytoplasm. Vacuoles found in mercuric-nitric or picro-acetic preparations are filled with yolk or oil in Meves' preparations or in the living *Platynereis* egg (*cf.* Wilson, '98, on the cytoplasmic structure of eggs, including that of *Nereis*).

The egg of *Platynereis*, as compared with that of *Nereis* fixed with the same methods, does not show so clearly the radial striation in the cortical layer or the homogeneous yolk spheres.

Ten minutes after laying the germinal vesicle is breaking down and maturation asters, formed outside its wall, are pushing into its substance. The deepest of the cortical alveoli are often still unemptied; the whole process of jelly extrusion can easily be followed from its beginning in inseminated eggs. On one or between two of the apices of the wavy vitelline membrane the spermatozoon is found attached by its perforatorium. Sperm head, middle-piece, and tail are readily distinguished (fig. 2).

Fifteen minutes after laying, the cortical jelly has been wholly extruded (fig. 3) and the first maturation spindle formed, with the chromosomes in late prophase. The endoplasm, with the extra-chromatin substance of the germinal vesicle, imbeds the spindle. *In toto* mounts of the egg at this stage, as is true of the *Nereis* egg, give no view of the spindle. One sees only a deeply stained core of substance which incloses the spindle. The egg is irregular in shape and the vitelline membrane is closely applied.

The spermatozoon is visible on the membrane (figs. 3 and 4) above a group of granules similar to those more thinly scattered throughout the periphery of the egg. These granules are markedly like those described by Meves and are doubtless 'mitochondria;' but in *Platynereis* they cannot possibly have the significance that Meves ascribes to them in the eggs of various forms. The granules appear massed beneath the point of sperm entry, but these masses assume no definite form. I have purposely figured those that give the nearest approach to cone formation (figs. 3, 4, and 5). A slender strand of cytoplasm may extend toward the membrane just below the perforatorium.

The granules in the region may appear as a disc, but never as a retracted cone, as in *Nereis*. The cortical breakdown has released the close application of the yolk spheres to the inner cortical margin; they are now irregularly spaced and among them lies the granular cytoplasm.

The figures (2 to 5) also give good pictures of the spermatozoa. They are much like the living spermatozoon. The head is almost spherical, the perforatorium a large blunt cap; the middle-piece and tail are often clearly defined.

2. *Penetration of the spermatozoon.* The penetration of the sperm head begins at twenty to twenty-six minutes after laying (*cf.* *Nereis*, forty-five minutes after insemination). The first maturation spindle, in the metaphase, is oriented in the polar plane of the egg; the inner endoplasmic mass which incloses the spindle is, at this stage, triangular in section; the outer aster of the spindle is near the apex of the triangle. The base of the triangle is less blunt than in previous stages and reaches farther outward along a radius of the egg. The various stages of penetration are shown in figures 6 to 18. The sperm substance enters the egg as a slender black thread, which gradually increases in size at its inner end. The sperm head, in my preparations, is usually homogeneously black, but often the external bulb is not so dark; or lighter areas appear along the entering thread; (particularly figs. 10, 11, 12, and 14). Often, especially in sections stained for twelve hours only, in stages just after the attachment of the perforatorium to the cytoplasm, the head appears, not as a homogeneous chromatin mass, but as a slightly differentiated body. One gains, therefore, the impression that the spermatozoon *flows* into the egg (*cf.* Koltzoff and Lillie, who, with different methods, find *Nereid* spermatozoa extremely ductile).

Cytoplasmic changes due to sperm entry are clearly marked during the later stages of penetration; striae appear in the cytoplasm around the entering spermatozoon, the area stains more deeply, and a projection from the endoplasmic mass reaches out toward the point of sperm entry (see figs. 14 to 17) (*cf.* on these points, Foot, Gardiner, Vedjovsky, Jenkinson, and Lillie, '12).

As the head is drawn into the egg, the inner bulb turns with its growth. Finally, the portion forming the external bulb is engulfed. The middle piece and tail, as in *Nereis*, never enter the egg (fig. 17). They may often be found in sections outside the membrane after penetration of the sperm head (see figures).

I have never found the spermatozoon in the *Nereis* or in the *Platynereis* egg at the time or in the form figured by Wilson ('96 and '00).

Does the sperm head rotate? I could not positively determine the rotation of the sperm head in the egg of *Platynereis*. In the first place, a definite cone organ, like that of *Nereis*, is lacking, and secondly, the middle-piece does not enter the egg. The history of the sperm penetration is known practically for every minute from entrance to pronuclear copulation. Meves' fixation alone was not depended upon. The Bouin preparations gave results much like those of Bonnevie's with picro-acetic mixtures on the *Nereis* eggs. While absolutely worthless for cytoplasmic detail, they were helpful in determining the structure of the sperm nucleus after penetration. The evidence favors rotation; the turning of the inner sperm bulb (fig. 17) and the position of the long axis of the sperm and aster as often found at right angles to the radius of the egg (fig. 22).

The sperm aster does not arise until the nucleus is beyond the yolk region (figs. 14 to 22). Within the endoplasm, the aster once formed, quickly divides equally, but the amphiaster does not long retain its equal poles, for one sperm centrosome and its aster gradually dwindle in size. Rays arise between one or both of the sperm centrosomes and the inner centrosome of the maturation spindle, thus forming a secondary spindle. The sperm nucleus lies nearer the larger sperm centrosome (see figs. 20 to 25).

3. *Copulation of the germ nuclei.* The egg chromosomes, after the formation of the second polar body, form fourteen chromosome vesicles which fuse to establish the egg nucleus (figs. 26, 27), all vestiges of the egg aster disappearing. The sperm nucleus enlarges as its asters become smaller. At the time of apposition, but one sperm aster is found (fig. 28). I believe that one sperm

aster begins to wane soon after the formation of the homodynamic amphiaser and finally disappears. One aster can always be found (fig. 29). The opposing nuclear membranes break down and one nucleus forms with the single sperm aster. Soon a small aster appears on the nuclear membrane (fig. 30), the nucleus breaks down, and the heterodynamic first cleavage spindle forms.

3. DISCUSSION

The case of *Nereis* and *Platynereis*, with respect to the entrance cone offers an interesting parallel with that of *Toxopneustes* and *Arbacia* (Wilson and Mathews). In both *Nereis* and *Platynereis*, however, the middle-piece is left outside the egg. The absence of cone-organ in *Platynereis* makes the question of rotation obscure, whereas in *Nereis* the evidence is indisputable.

Bonnevie ('08), in her paper on *Nereis*, has mentioned certain cytological differences between the "large and small varieties" of *Nereis* eggs. As indicated above, the time of sperm entry is earlier in *Platynereis*. It is also true that the polar bodies are formed earlier, the first cleavage is earlier, and the subsequent rhythms are faster, so that the larval stage is reached earlier.

So far as both *Nereis* and *Platynereis* are concerned, the rôle of the middle-piece or its contained centrosome, as the chief actor in fertilization, is wanting. There are spermatocytes with intra-nuclear centrosomes² (see Julin on *Styleopsis*). But if this hypothesis be postulated (*cf.* Packard in 1914) for *Nereis* sperm, this next step, as was pointed out by Lillie in 1912, should also be taken: the centrosome gradient must be quantitatively different from its base at the middle-piece to the tip of the sperm head: "If intra-nuclear centrosomes are the causes of the formation of the sperm aster, not only must they exist at every level, but also (that) they must decrease in size from the base to the apex of the sperm nucleus!"

² See also Hegner and Newman for intra-nuclear centrosomes in oocytes.

According to Schaxel, the middle-piece does not enter the egg of echinoderms. Meves will not admit this for echinids and doubts that in *Nereis* the middle-piece is left outside the egg while denying the centrosome the chief part in fertilization. In *Platynereis*, as in *Nereis*, by diverse methods it can be shown that the middle-piece does not enter the egg. We are thus forced to conclude that, whatever its rôle, the middle piece in *Platynereis* can play no part, either in heredity or through a centrosome in the dynamics of fertilization.

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DESCRIPTION

All figures were drawn with the camera lucida with Leitz $\frac{1}{2}$ oil immersion objective and No. 5 ocular, except where otherwise stated. All figures from sections of inseminated eggs of *Platynereis megalops*. All sections from eggs killed in Meves' fluid and stained in iron haematoxylin.

PLATE 1.

EXPLANATION OF FIGURES

1. Section of an unfertilized ovocyte. The oil drops are a delicate brown, the granular yolk spheres very lightly stained. The cortex is intact.

2. Ten minutes after laying. The cortex is partially reduced. The head, middle-piece, and tail are clearly shown.

3 to 5. Fifteen minutes after laying. The granules are massed below the point of sperm attachment; the perforatorium is still attached to the membrane 6 to 8. Twenty minutes after laying.

6. The perforatorium is touching the cytoplasm. The granular mass has disappeared.

7. The perforatorium is in the cytoplasm.

8. A somewhat tangential section, showing the very beginning of penetration.

9 to 13. The penetration stages, twenty-five minutes after laying, mesophase of the first maturation division. The figures show that there is no constant disposition of granules at the point of sperm entry—certainly nothing of the nature of a cone, as in *Nereis*.

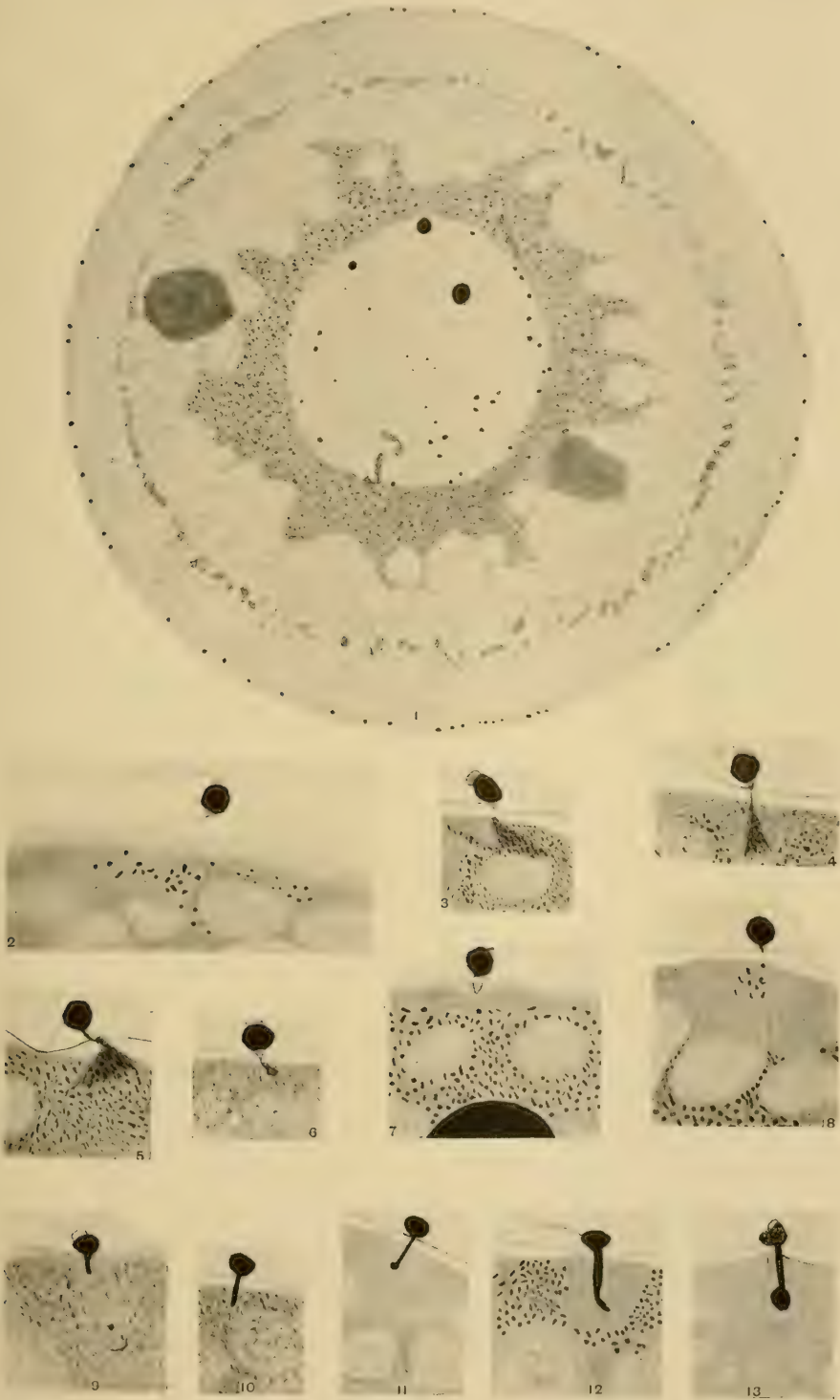


PLATE 2

EXPLANATION OF FIGURES

14, 15. Penetration stages, twenty-five minutes after laying. Note the turning of the inner sperm bulb.

16 to 18. Twenty-seven minutes after laying. The middle piece is shown in fig. 17. In 16 and 18 the middle-piece was found in adjacent sections.

19 and 20. Thirty minutes after laying, telophase, first maturation division. The sperm head is still within the zone of oil drops and without an aster.

21 to 22. Thirty-two minutes after laying; early prophase, second maturation division.

21. The sperm head is at right angles to a radius of the egg, the aster forms around the granule at the tip of the sperm head.

22. Formation of sperm aster within the endoplasm.

23. Thirty-five minutes after laying. The amphiaster is in contact with the egg aster. The spermatozoon is in an adjacent section.

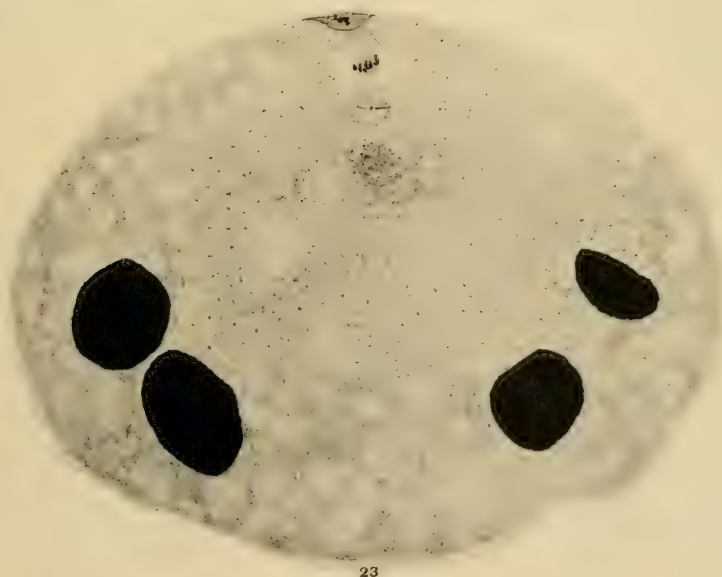
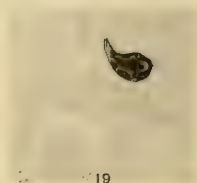
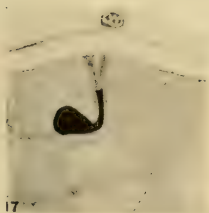


PLATE 3

EXPLANATION OF FIGURES

24 to 30; oc. 1, $\frac{1}{12}$ oil im. Later stages, showing marked inequality of sperm asters. Note relation of the spermatozoon to the larger aster.

25a and b. Forty minutes after laying.

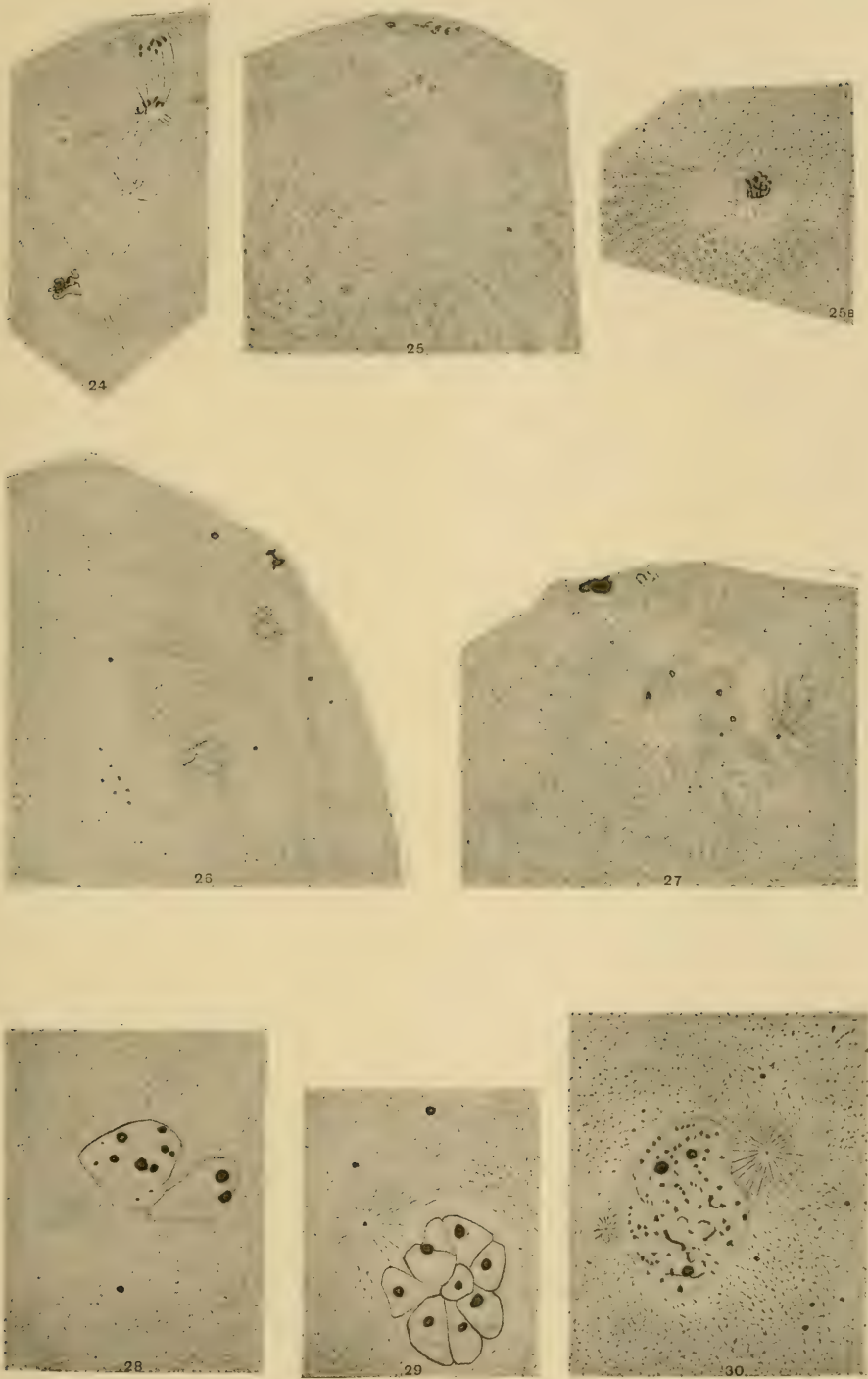
26. Forty-six minutes after laying. The egg aster is degenerating.

27 to 29. Copulation stages.

27. The smaller sperm aster could not be found. Two egg and three sperm nuclear vesicles are shown.

28 to 29. Formation of the male and female nuclei.

30. Origin of the first cleavage spindle.



CILIATED PITS OF STENOSTOMA

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University of Virginia

FOUR FIGURES

The material for this paper was found upon glea and sediment deposited upon submerged leaves and twigs taken from both near the surface and the bottom of pools in the vicinity of the University.

The animals are about 1 to 2 mm. long and 200 micra broad at the widest region. The body is oblong, spindle shaped, and is widest in the region of the mouth; it is grayish white and its epidermis, which contains rhabdites, is thickly covered with short cilia. The mouth is on the ventral side, about 150-200 micra posterior to the anteriorend. The urinary system consists of a single nephridium.

The ciliated pits are by far the most striking organs of the body which are externally visible. These are small invaginations in the epidermis which are located lateral-dorsally about 100 micra from the anterior end of the body. Their shape is, in general, similar to small sacs, but their lateral walls are highly contractile so that the pit may be made to assume the form of a deep cut or that of a shallow, concave disc. The ciliated pits have a diameter of about 50 micra and a depth of about 40 micra.

This paper is concerned with the minute anatomy of the ciliated pits and their development. In order to carry out such a study the animals were fixed in Flemming's stronger fluid, which consists of 15 parts 1 per cent chromic acid, 4 parts 2 per cent osmic acid, 1 part glacial acetic acid; time of fixing 25 minutes. Chrome-aceto-formaldehyde, hot and cold solutions of aceto-sublimate, and Zenker's fluid were all tried, but without success,

either causing great distortion or disintegration of the animals. The worms were cut into sections, some three and some five micra thick and the sections stained with iron haematoxylin and counter stained with Bordeaux red. Macerations stained with such intra vitam stains as Wright's stain and methylen blue were very valuable in corroborating results.

HISTOLOGY OF THE PIT

The histology of the pit involves an understanding of the epidermis. The epidermal cells of the animal have the power to secrete a protective mucus-like substance. That such is the case can readily be seen by placing the animal in an abnormal solution of not too rapid killing power. The animal will at once enshroud itself in a thick sheath of protective mucus within which it swims around. Such a phenomenon will be more fully described in a later part of this paper.

The pit is associated with a region of the central nervous system known as the ciliated pit-ganglion. The pit, as well as this ganglion, is a modified region of the general epidermis. The marginal walls of the pit are formed by cells transitional in structure between the general epidermis and the low cells at the bottom or fundus of the pit. As the invagination which forms the pit takes place this transition takes place until there is a layer of low, small epithelial cells lining the fundus of the pit. The boundaries of these fundus cells are less pronounced than the boundaries of the general epithelial cells and their nuclei less frequent. In certain regions of this lining of the fundus the few nuclei which are present are indefinitely placed, which fact suggests that there is no basement membrane.

On the exterior of the body, lying close upon the fundus of the pit, is a homogeneous mass of mucus-like substance. The marginal walls of the pit are thickly covered with cilia which appear to be longer than the cilia of the general body epithelium, but no cilia at all were found upon the low cells lining the fundus nor were any seen projecting above the homogeneous mass of mucus-like substance.

The ciliated pit-ganglion is by far the most conspicuous feature of the pit. It is located just within the body and lying around the base of the ciliated pit in a cup-like manner. The cells of this structure are only indistinctly separated from those of the dorsal ganglion or 'brain' by a few muscle fibers and have the same characteristic, granular nuclei as those of the 'brain.' Some of these ciliated pit-ganglion cells are seen to send processes through the epithelium of the fundus which lies in contact with the homogeneous body of mucus-like substance. These we take to be sensory rods of highly special nature which enable the organ to detect very slight changes in its surrounding medium. These sensory rods are shown clearly, as dark blue structures, in intra vitam staining with Wright's stain and in many of the regular sections. Figure 4 shows such a section. The fact that the ganglion cells arise from the epithelial cells which line the fundus of the pit also supports the idea that these processes are left behind by the cells as they migrate inward to enter into formation of the ciliated pit-ganglion.

Rightful interpretation and appreciation of the above statements will only be obtained through a study of the origin of the ciliated pits.

THE ORIGIN OF CILIATED PITS

The origin of the ciliated pits can readily be studied in specimens which are dividing. The first appearance of the pits is seen in two sharp, abrupt depressions of the epidermis, one on each side of the animal. From the bottoms of the depressions (i.e., the region which will be the fundus of the new pit) the cilia disappear. Figure 1 shows an early pit in this stage of development. Ventral to this abruptly lowered region a crowded mass of mesenchymal cells is formed which represents the anlage of the 'brain.'¹

¹ We are indebted to Prof. Böhmig, through the kindness of Prof. L. von Graff, for the following quotation from page 34 of O. and R. Hertwig's *Die Coelomtheorie*, Jena, 1881. In regard to the Platyhelminthes they say: "In der Abteilung stammt wahrscheinlich der motorische Teil der Centralorgane des Nervensystems im Anschlusse und die Muskulatur aus dem Mesenchym, der sensorielle Teil im Anschlusse an die Sinnesorgane aus dem Ektoderm."

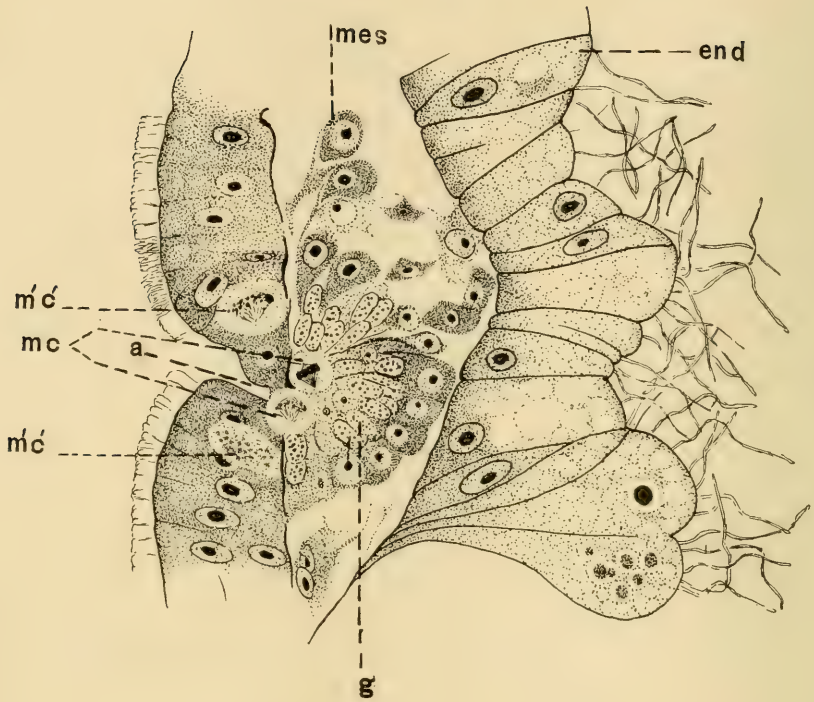


Fig. 1. An early stage in the formation of the ciliated pit and its ganglion. (end.) Endoderm or wall of enteron. (g) General epidermis lowered at (a) to form the rudiment of the fundus of pit. Note absence of cilia in this lowered region and migrating mitotic cells (mc, m'c'). (mes) Mesodermal cells crowded about the forming ciliated pit-ganglion (g). $\times 1500$.

This sharp depression is already a rudimentary pit with its non-ciliated fundus and its ciliated marginal walls but lacks a ciliated pit-ganglion. At this time, about the fundus of the rudimentary pit mitoses arise which send into the mesenchymal space between the fundus and the anlage of the 'brain,' which has already been formed, a proliferation of cells which radiate from beneath the developing fundus of the pit. This mass of cells is the beginning of the ciliated pit-ganglion.

Thus there are established at the outset two parts of the ciliated pit. a) The epithelium of the pit; b) The rudiments of a ciliated pit-ganglion.

The epithelium of the growing pit is extended as a region which is morphologically different from the general epidermis in that its cells are lower and are repeatedly dividing to yield additional cells to the formation of the ciliated pit-ganglion; also in that the cells which line the fundus of the pit have irregularly placed nuclei, and have lost their cilia, while the cilia on the marginal cells have become longer than the cilia on the general body epithelium. These characters are shown in figures 2 and 3. Up until this stage in their development the cells of the pit-epithelium retain their power to elaborate rhabdites, as is illustrated in figures 2 and 3.

As the pit grows larger no rhabdites are to be found in the epithelium of its fundus. But before these rhabdites have totally disappeared the formation of a peculiar body is started, which in the mature pit is a highly refractive, homogeneous layer, which Ott ('92)² has called the 'homogeneous mass.'

The nature of this 'homogeneous mass' can best be arrived at by observation of the specimens during fixing. As an animal lies in contact with the slide, if it be fixed by dropping the fixing fluid upon it, it will adhere to the slide on account of the protective discharge thrown out by the cells of the general epithelium. To avoid such trouble it was necessary to apply the fixing fluid with a dash as the animal swam around in a small drop of water on the slide. Thus any adhesions which would injure the fixed specimen were avoided. The details of this trouble can be plainly observed under the binocular microscope. If an entire specimen be treated with methylen blue, a protective blue sheath of mucus with imbedded rhabdites (stained deep blue), will be seen to be formed around the animal. If this sheath be removed from the specimen and the stain again applied the epidermal cells fail to respond and the protective sheath is not formed the second time.

Thus it is evident that in an effort to protect itself the epidermis not only discharges rhabdites but also a mucus which stains with methylen blue less deeply than the rhabdites. Now, since

² Ott; Jour. Morph., vol. 7, 1892.

in the fundus of the mature pit there are no rhabdites, it is suggested that, as the cells of the developing epithelium are physiologically differentiated, they lose their power to elaborate rhabdites and develop a greater capacity to secrete a permanent, refractive, mucus-like glea which protects the greatly exposed and extremely sensitive fundus of the pit. So we draw the conclusion that the only difference between the mucus secreted by the cells of the general epithelium and the mucus which composes the 'homogeneous mass' of the ciliated pit is that the 'homogeneous mass' is permanent, perhaps more dense, and withstands the action of reagents better than does the temporary secretion of the general epithelial cells. We have made comparison of these two substances by staining with methylin blue, in which case they stain alike, both staining in living specimens a rather dark blue as contrasted with the intensely dark blue of the rhabdites. So much for the development of the epithelium of the ciliated pit and its secretion product.

DEVELOPMENT OF THE GANGLION OF THE CILIATED PIT

As has been stated previously in this paper, beneath the forming ciliated pit there is a mass of cells which we take to be mesenchymal in origin. This statement is made in abeyance since we are not concerned at present with the origin of the 'brain.' We have, however, been able to see that the 'brain' arises from these cells. But the important point which we endeavor to make is that the ciliated pit-ganglion has a distinct origin from the epidermis.

With the earliest formation of the pit-depression at the surface of the body there occur mitoses in its epithelium which send into the mesenchymal region a number of cells which locate themselves between the epithelium of the fundus and the mesenchymal cells which form the 'brain,' as shown in figure 2. There is, however, a distinction at the very outset between the cells of the 'brain' and those which are forming the ciliated pit-ganglion, as shown by the above figure. This proliferation of cells arising from the epidermis continues to grow with the development of the superficial part of the pit.

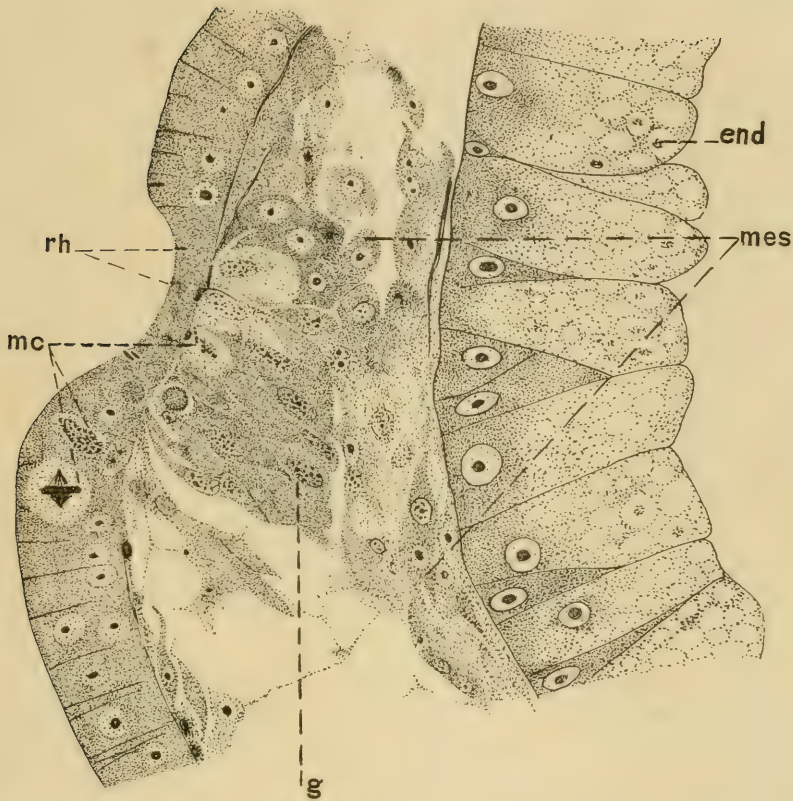


Fig. 2. Later stage of formation of ciliated pit and its ganglion. Note widened fundus with its low epithelium that yet has rhabdites (rh). Mitoses (mc) in region of fundus continue to be present. Ganglion (g) has enlarged. (mes) Mesoderm that develops into 'brain' and commissure. (end) Endoderm. $\times 1500$.

Throughout the growth of this ganglionic mass of cells their nuclei have a constant chromatin pattern. At the earliest and intermediate stages of the development of these cells their nuclei tend to be oval while their cytoplasmic bodies are more or less elongated, pyriform, or spindle shaped. Figures 1, 2 and 3. In the final stages of their development the cell bodies become less distinct until in the mature ganglion there is only a network of fibers or cytoplasmic strands supporting many spheroidal nuclei. Figure 4.

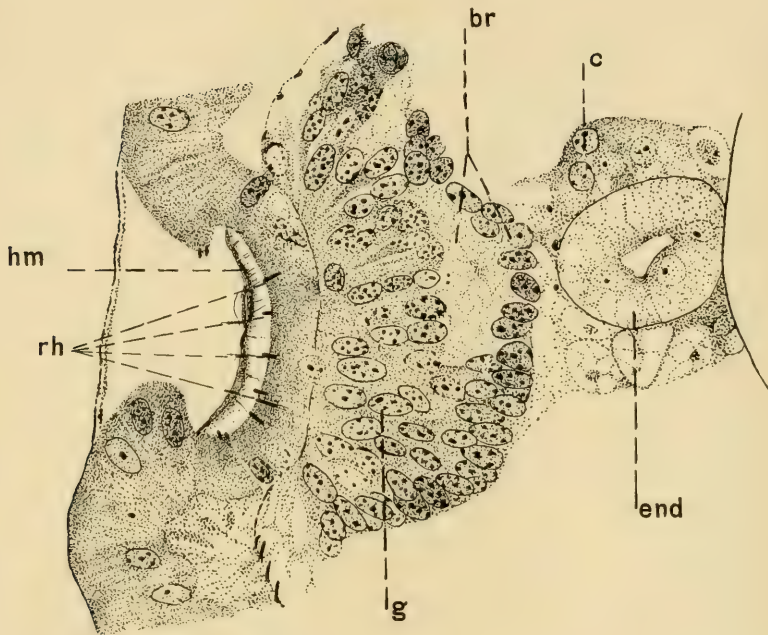


Fig. 3. Later stage in formation of ciliated pit and ganglion. Note appearance of 'homogeneous mass' (hm), with rhabdites (rh) yet present in the fundus epithelium. Ciliated pit-ganglion (g) has now fused with the fibrous part of the 'brain' (br); (end) Constricted enteron. (c) Commissure of 'brain' forming. $\times 1500$.

The interesting outcome of this development is a sensory epithelium from which many cells have retreated, leaving behind a low, secreting epithelium through which they leave elongated processes of themselves.³ Figure 2. These processes are the sensory ends of the ganglionic cells which have been described. Thus we have the development of a ciliated pit whose marginal cells are covered with extremely long cilia which may protect the delicate fundus against impacts of external objects by practically closing the mouth of the pit to any particles of matter which might enter and in any way injure the sensitive

³ It cannot be definitely stated that all of these ganglionic cells have such processes, since on account of the nature of the case, only a few such processes in each animal can be sectioned parallel to their axes.

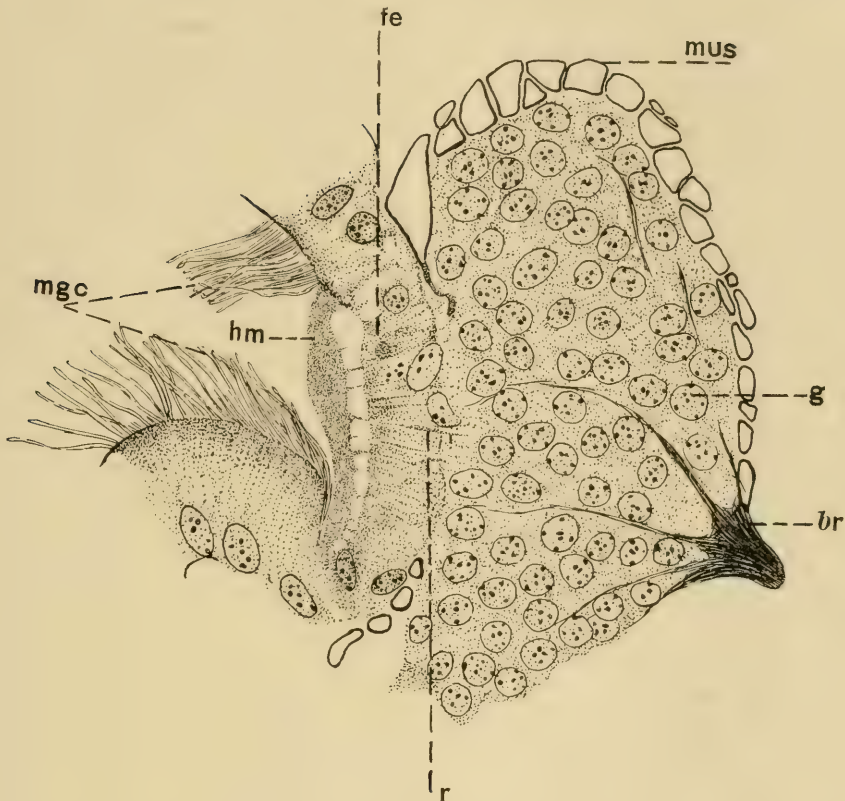


Fig. 4. Ciliated pit with its 'homogeneous mass' (hm); fundus epithelium (fe); marginal epithelium with its cilia (mgc), and pit-ganglion (g) well established; the latter receiving a bundle of fibres from 'brain' (br). (mus) Muscles. $\times 1500$.

base. Over the base is spread the 'homogeneous mass' elaborated by the epidermis of the fundus into which the sensory rods of the ciliated pit-ganglion cells extend and test the chemical nature of the water, conveying the sensations obtained to the pit-ganglion, which merges into the 'brain.'

In these results we have been able to confirm the description of Ott ('92) so far as the general structure of the pits is concerned, but have not, however, been able to agree with his description of the fundus of the pit. He says "the cilia of the

epithelial cells could be seen passing through the homogeneous mass," and that—"the cilia on the small cells at the base of the pit are like those of the epithelial cells of the integument except that they are much longer. They range from 8 micra to 15 micra in length."⁴ We have found that the cilia on the marginal walls of the pit are longer than those on the general surface of the body but have found in no case any suggestion of cilia related to the fundus of the pit or its 'homogeneous mass.' According to Ott,⁵ Landsberg says "that the bottom of each pit is covered with a thick layer of homogeneous substance which may be regarded as mucus. Below this is a thin layer of ciliated epithelial cells whose cilia project through the homogeneous layer. Next to this is a much thicker layer which is made up of mostly pyriform cells, although there are other histological elements scattered through it. Next to this layer is the ganglion which is connected with the nerve." This description is very much in accordance with our results with the exception of the statement that there are cilia on the low cells at the fundus of the pit. Ott says "There are three possible methods by which the three layers described by Landsberg might be produced: 1) By a division of the epithelial cells, 2) by a migration of cells from the brain ganglia to the walls of the pits, 3) by a migration outward of some of the epithelial cells to form a second outer layer. If a new layer of cells was formed by the first method we ought certainly to find numerous spindles vertice to the surface in every developing pit."⁶ Now we have assumed the first mentioned method of formation of the ciliated pit ganglion mainly on account of the fact that we have seen these numerous spindles as described by Ott (fig. 2). We find them in nearly every section that passes through the fundus and ganglion.

In one other respect our conclusions differ from a previous description of the general structure of the pits by von Graff ('13). He says "Das Nervensystem besteht aus zwei langgestreckten Hälften, deren jede durch eine swache Einschnürung

⁴ Jour. Morph., vol. 7, p. 291, 1892.

⁵ Same reference, p. 291.

⁶ Same reference, pp. 292-3.

in eine hintere, durch eine breite Kommissur verbundene und eine kleinere, vordere Partie zerfällt. *Die letztere bildet die beiden Grübschenganglien, in welche sich die Wimpergrübshen einsenken.*⁷ (Das Tierreich, s. 20.) According to our observations, the ciliated pit-ganglion does not arise from the 'brain' but arises independently from the epidermis.

In conclusion, it is interesting to observe the striking parallelism presented by this organ in its function and mode of origin with the olfactory organ of a vertebrate so far as its function (i.e., its function in the fish) and its mode of origin is concerned.

This organ functions as a tester of the chemical nature of the water which passes through or over it as do the olfactory organs of the fish. Moreover, this organ arises as a modified region or plate in the epidermis. Some cells of this plate sink beneath the base of the plate to form the ganglion of the pit. All this is closely analagous to the following description of the origin of the olfactory ganglion as given by Minot ('92).⁸

The ectodermal cells of the olfactory plate multiply, the karyokinetic figures being found next to the outer or free surface of the layer; the cells thus produced assume the appearance of medullary neuroblasts and at four weeks are found migrating toward the mesenchymal surface, so that the base of the layer of the olfactory ectoderm becomes crowded with nuclei; the protoplasm of these neuroblasts is collected on one side of the nucleus in a pointed mass; the cells now grow forth from the ectoderm and constitute the anlage of the ganglion between the ectoderm and the brain. (P. 637.)

CONCLUSION

The ciliated pit and its ganglion in this flatworm arise from the general epithelium in a manner closely analogous to the mode of origin of the olfactory epithelium and olfactory ganglion of the vertebrates.

⁷ Italics our own.

⁸ Minot; Human Embryology.

THE DEVELOPMENT OF THE ALBINO RAT, MUS NORVEGICUS ALBINUS

I. FROM THE PRONUCLEAR STAGE TO THE STAGE OF MESO- DERM ANLAGE; END OF THE FIRST TO THE END OF THE NINTH DAY

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THIRTY-TWO FIGURES

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INTRODUCTION

The early developmental stages of placental mammals, embracing the stages of sex cell maturation and fertilization, of segmentation, of blastodermic vesicle and germ layer formation, though subject of numerous contributions extending over many years, have in no form been completely investigated. The literature dealing with the phenomena of maturation and fer-

tilization as observed in placental mammals has in recent years been enriched by a number of studies to the extent that for certain of the mammals—bat, rabbit, guinea-pig, mouse, and rat—the data at hand are sufficiently complete to enable a clear and comprehensive presentation, based on observed facts, and permit of comparison with similar phenomena as observed in other vertebrate and invertebrate forms. As concerns the process of segmentation in placental mammals, there are still lacking sufficiently comprehensive observations embracing a number of forms to enable a clear and succinct presentation of the rate of blastomere formation, the cytomorphosis of the cells, and of the relative position of the several segmentation stages in the genital tract. This is no doubt owing to the difficulty of obtaining the necessary material timed so as to admit of proper staging, and the impossibility of making extended observations on living material. Our knowledge of the phenomena of blastoderm vesicle formation, though comprehended in its general phases, is lacking in detail, except for a very limited number of forms. The process of germ layer formation is of such fundamental importance to a clear comprehension of later developmental stages, both in phylogeny and in ontogeny, that a brief account of observed facts in any one form may not be regarded as wholly without value.

Opportunity presented itself, while stationed at The Wistar Institute of Anatomy and Biology, to collect and fix an extended series of embryological stages of the albino rat. This material has proven sufficiently comprehensive to enable a presentation of the several developmental stages of this mammal, beginning with the pronuclear stage and extending to the stage of the anlage of the mesoderm. For this period, which extends to about the tenth day after insemination, only very few of the essential stages are lacking, though for certain of the stages confirmatory preparations would have been desirable. The material at hand, however, seemed sufficiently complete to present a connected account of the stages it is hoped to cover. The embryology of allied forms, especially of the mouse, has received much more extended study than has that of the rat, though the develop-

ment of the rat has received especial consideration by Fraser, Christiani, Selenka, Duval, Robinson, Widakowich, and as concerns maturation and ovulation, by Sobotta and Burekhard, Kirkham and Burr. The pertinent literature will be considered in connection with the presentation of my own results.

MATERIAL AND METHODS

The material on which this investigation is based was obtained from albino rats (*Mus norvegicus albinus*, Donaldson)¹ taken from the extensive rat colony of The Wistar Institute of Anatomy and Biology. The experience gained in the breeding, feeding, and growth experiments, extending over many years, conducted by Donaldson and his associates and resulting in numerous excellent publications, was at my disposal while collecting this material. The material used was all carefully timed, so that sequence of stages was obtained with some degree of certainty. With care and experience, it is possible to regulate and observe insemination, so that stages may be approximated quite accurately. Kirkham and Burr state that "on several different occasions we have observed actual pairing" of the albino rat. Widakowich states that he was unable to obtain accurate data as to the age of the embryos except by observing coitus. According to this observer, a female rat permits many males to copulate in the course of several hours, receiving males 30 times or oftener, when suddenly she drives them away. Sobotta and Burekhard, on the other hand, admitted males a few hours after parturition, depending on the fact that many mammals ovulate soon after parturition. Though attempts were made, they were unable to observe pairing, and they state that the 'Dieners' charged with the care and feeding of the rat colony were only seldom able to observe attempts at pairing. At The Wistar Institute no difficulty is experienced in pairing albino

¹ Melissinos and Widakowich state having used as material the albino rat, variety *Mus rattus albinus*. Donaldson has conclusively shown, that by reason of physical characters—blood crystals, shape of the skull, etc.—the albino rat kept as pet or laboratory animal cannot be *Mus rattus albinus*, but must be *Mus norvegicus albinus*.

rats. Dr. J. M. Stotsenburg, to whose experience and careful records I am greatly indebted for the trustworthiness of the material collected, made use largely of females who had born one litter. Pairing was seldom attempted a few hours post partem, as was done by Sobotta and Burekhard, but usually about 30 days after the birth of a litter, which may have been nursed or otherwise disposed of. The great majority of females used in pairing were at the time free from 'domestic cares.' The females employed were kept in separate cages for some time before giving birth to young and until the time of mating. About 30 days after the birth of a litter, a male was placed in the cage with the female. If the female was in heat, copulation usually took place soon after. The male was left with the female for an hour to an hour and a half, during which time several pairings would occur, and at the end of which time the female would try to hide from the male, climb the side of the cage and defy him with her teeth. The male albino rat is not prostrated by the sexual act, the same male serving for several successive copulations. In case the female was not in heat, this soon became evident and the male removed, to be again placed into her cage 24 or 48 hours later. The time when the copulation was first observed was noted on the card attached to the cage and gave the time from which the age of the embryo or respective stage was reckoned. The time given is, therefore, that of 'insemination,' a term which Long and Mark have introduced to indicate "the introduction of the male sexual elements into the genital tracts of the female by the act of coitus or otherwise." This time could be accurately noted, while 'semination' which "applies to the access of the spermatozoa to the eggs in the oviducts, the coming into contact of the male and female reproductive cells" can not be accurately timed. The success attained in pairing albino rats as above stated, obviated the necessity of depending upon chance material or resorting to 'artificial insemination' as described for the mouse by Long and Mark. I am at loss to understand why Widakowich should regard the age determinations of Sobotta and Melissinos (mouse embryos) more accurate than his own, reckoned from the time

of observed coitus. The slight though observable variation in the rate of development in a series of ova of the same animal, more marked when supposedly similar stages of several animals are investigated, precludes the accurate timing of stages.

As fixing fluids, there were used Zenker's fluid, sublimate-alcohol, Flemming's fluid, Bouin's fluid, and Carnoy's fluid. After a few trials, all were discarded in favor of Carnoy's fluid, prepared by mixing 6 parts of absolute alcohol, 3 parts of chloroform, and 1 part of glacial acetic acid. This somewhat illogically compounded fluid penetrates rapidly and does not cause shrinkage. Tissues are fixed in it for several hours, then washed in several changes of absolute alcohol in which it has been my custom to store the tissues. The following procedure was practiced in all stages up to about 12 days after insemination: The animals were anaesthetized and the head severed from the body, to admit of free bleeding. The rat was then fastened to a board, and thorax and abdomen opened by a mid-sagittal incision, the abdominal walls pinned back, and the intestine elevated toward the thorax. With as little manipulation as possible, the ovaries were separated from their attachment, the mesometrium cut, the uterine horns elevated and the vagina severed. The whole genital tract was then placed on a clean slide and arranged in approximately normal position. Slight tension was maintained by tying a thread to the connective tissue removed with each ovary and bringing the threads along the reverse side of the slide and tying them to the vagina. If the slide is clean, the mesometrium of each uterine horn may be spread out evenly and caused to adhere to the slide. Ovaries, oviducts, and uterine horns may thus be spread out in normal position and each uterine horn fixed as a straight tube. When thus arranged on the slide, the preparation was placed in a relatively large quantity of Carnoy's fluid, fixed, and then transferred through several absolute alcohols. For nearly all the material used in this study, the method of fixation was as here given. In the earlier stages of material collection, attempts were made to obtain segmentation stages in warm normal salt solution. Several were thus obtained and were used to control

the observations made on sections, as will be discussed later. By cutting the oviduct at about its middle, freeing it from its mesosalpinx and cutting the uterus about 1 cm. below the insertion of the oviduct, a pipette fitted with a rubber bulb and filled with warm normal salt solution can be inserted into the uterine cavity and moderate pressure made. It is usually possible to wash into a watch crystal a certain number of the contained segmenting ova. Before reading the article by Widakowich, essentially the same method as employed by him, for isolating implanted blastodermic vesicles was developed. This may be quite readily done after fixation in Carnoy's fluid and teasing under a stereoscopic binocular. Vesicles sectioned *in situ*, however, gave on the whole more satisfactory results, so that teasing out implanted vesicles was not resorted to.

The fixed tissues were imbedded in paraffin, using xylol as a clearing fluid. For stages including those falling within the period ranging from the first to the fourth day after insemination, the ovary and oviduct to its insertion in the uterus, were embedded *en masse*. For stages falling within the period of fifth to sixth day after insemination, the uterine horns were divided into segments measuring about 1.5 cm., and sectioned parallel to the plane of the mesometrium. For later stages, after the enlargements in the uterine horns are distinctly evident, these were removed and cut severally in the three planes. The great majority of the sections were cut at a thickness of $10\ \mu$; certain ones at a thickness of $5\ \mu$; a few at a thickness of $7\ \mu$. The sections were fixed to the slide by the water-albumen method. The great majority of the series were stained in hemalum, counterstained in Congo red. This solution, which presents certain advantages as a counterstain for embryologic tissues, is prepared as follows: 0.5 gms. of Congo red (Grübler) is placed in 100 ccm. of distilled water and the water brought to boiling. This should give a clear solution. Before cooling, add 100 ccm. of distilled water and 10 ccm. of absolute alcohol. The Congo red solution thus prepared may be kept many weeks. After staining the series in the usual way in hemalum, they are differentiated in acid alcohol, and passed through several washes of 'tap water' into distilled

water. They are then stained in the Congo red solution, which may be diluted with distilled water about five times. With the diluted solution, the counterstaining requires one to two hours. The sections are then rinsed in distilled water, differentiated in 80 per cent alcohol, dehydrated, cleared, and mounted in damar. Certain of the series were stained in Heidenhain's iron-hematoxylin and counterstained in Congo red. The drawings accompanying this contribution were nearly all drawn on coarse 'Ross board,' with the aid of the camera lucida at a magnification of 1000 diameters, using pencil and India ink. Such drawings admit of liberal reduction, and give a detail not readily obtained otherwise. Free use has been made of the Born method of reconstruction, especially for earlier stages. The majority of the models thus obtained are here reproduced.

I desire to express my sincere thanks and appreciation of the very material aid given me by Mr. Wayne J. Atwell, then Assistant in the Department of Histology and Embryology of the University of Michigan, in the making of the reconstructions of the oviducts included in this account.

OVULATION, MATURATION, AND FERTILIZATION

When this study was projected, it was the purpose to begin it with the stages of maturation and fertilization. During the time of material collection, there appeared the contribution of Sobotta and Burekhard: "Reifung und Befruchtung des Eies der Weissen Ratte," covering these stages fairly completely. Duplication of their work did not seem necessary, so that my own studies begin with the pronuclear stage, to which stage the above mentioned investigators had carried their observations. Therefore, as concerns the process of ovulation, maturation, and fertilization as observed in the albino rat, I am confined for my data to the literature; from which a brief résumé is here made.

The normal gestation period for non-lactating albino rats may be roughly estimated as from 21 to 23 days. As has been shown by King, the period of gestation of lactating albino rats varies

from a minimum of 24 days to a maximum of 34 days. The average number in a litter is six. In lactating females suckling five or less young and carrying five or less young, the period of gestation usually does not exceed 23 days and may thus be considered as normal. In lactating females suckling five or less young, while they are carrying more than five young, the period of gestation may be prolonged from one to six days. In lactating females suckling more than five young, the period of gestation is always prolonged, and may be prolonged to a maximum of 34 days. Daniel's studies on the white mouse lead him to formulate the following law: "The period of gestation in lactating mothers varies directly with the young suckled." Such exact relation between the number of young suckled and the extent of the prolongation of the gestation period was not observed by King for the albino rat.

In the albino rat, ovulation occurs spontaneously and is not dependent on copulation, which act, however, may precede or follow ovulation. Kirkham and Burr state that ovulation usually occurs about 24 hours after parturition and that the developing ova can be traced in the ovary through the two oestrus cycles preceding their discharge. Long, in his study No. 3, by Mark and Long, finds that ovulation must occur in the albino rat on an average not less than 18 hours after parturition. Sobotta and Burekhard state that ovulation always occurs within 36 hours post partem, though at very variable periods, often only a few hours after the completion of parturition; again, much later. A second ovulation period apparently occurs some 30 days post partem, as would appear from the successful pairings conducted by Dr. Stotsenburg. This agrees with the observations of Melissinos, who found that pairings were more numerous when attempted 29 days after parturition, than when attempted 20 to 21 days after parturition, as practiced by Sobotta. Semination probably takes place in the ampullar portion of the oviduct. Relatively few spermatozoa enter the oviducts and Sobotta and Burekhard estimate that the life of the spermatozoa in the genital tracts of the albino rat is only about 10 hours.

The phenomena of maturation and fertilization in the albino rat have been carefully studied by Sobotta and Burekhard, from whose account the following brief summary is taken: The behavior of the ovum of the albino rat with respect to the formation of polar bodies is very similar to that of most other mammals studied. The first polar body is given off within the ovarian follicle, the second in the oviduct and only after semination. The first maturation spindle, developed from the nucleus of the oocyte of the first order, forms usually immediately after parturition. Kirkham and Burr state "it is usually formed less than 24 hours after parturition." It is short and broad, with the chromatin scattered. The first maturation spindle lies near the center of the ovum, then passes toward the surface assuming a tangential position, and only with the beginning of metakinesis, takes a radial position. The chromosomes of the first maturation spindle, estimated as numbering 16, appear in the form of modified rings, which are divided transversely across to form short rounded rods with a longitudinal direction in the diaster stage. The first polar body is formed in the ovarian follicle and appears to be relatively large. It is evident only in the ovarian ovum, and appears to be lost soon after its formation. Its fate is doubtful. The first polar body is nearly always missing in tubal ova. Kirkham and Burr state that "the rare occurrence of the first polar body associated with the egg in the tube is to be attributed to its rapid disintegration, which begins as soon as it is formed, and may lead to complete disappearance before ovulation occurs." The second maturation division begins immediately after the completion of the first, without an intervening resting phase. The spindle formed is narrower and longer than the first, with the chromatin massed. In its monaster stage, it lies in a tangential position, with the chromatin in diads, and with the lines of division at right angles to the axis of the spindle. The appearance of the second maturation spindle in the monaster stage marks the end of the maturation phenomena in the ovary. The monaster stage of the second oocyte division was not observed in the ovary by Sobotta and Burekhard, but was seen by Kirkham and Burr. The first

division Sobotta and Burekhard regard as a reduction division, a heterotypic longitudinal division; the second as an equatorial division, a homeotypic longitudinal division. Ovulation probably occurs during the monaster stage of the second maturation division.

The tubal ova are surrounded by a relatively thin oolemma to which are adherent a variable number of discus cells. They are smaller than the ovarian ova; the latter measuring $60\ \mu$ to $65\ \mu$, the tubal ova $55\ \mu$ to $60\ \mu$. The recently discharged tubal ova are to be found in the distended ampullar portion of the oviduct, where they are found clumped together surrounded by discus cells. Semination takes place in this region. The spermatozoa usually enter while the tubal ova are in the monaster stage of the second maturation division, after which metaphase begins. The second maturation spindle assumes a radial position in the metakinetic phase. The second polar body is smaller than the first, and usually lies compressed between the oolemma and the ooplasm, and is evident during fertilization and segmentation. The spermatozoan head penetrates the thin oolemma and the ooplasm; the long middle piece and tail following the head into the ooplasm, as has been shown by Coe, and Kirkham and Burr. The long middle piece, soon after penetrating the ooplasm, presents an increase in stainability, and its spiral thread becomes evident. The spiral thread, as Duesberg has shown, has its origin in the mitochondria of the spermatid. It may be, therefore, that the male sexual cell introduces mitochondria to the egg cell at the time of fertilization. Some little time after the penetration of the sperm head, this enlarges and becomes vacuolated, and diplosomes with polar rays become evident. As the sperm head begins to metamorphose, tending to the formation of the male pronucleus, the chromosome group of the dispirome of the second maturation spindle, undergoes metamorphosis to form the female pronucleus. This enlarges rapidly to form a vesicular nucleus which lies free in the ooplasm, while the metamorphosing male pronucleus, usually smaller, is accompanied by a deeply staining thread-like structure, derived from the middle piece. The centrosomes of the

first segmentation spindle are by inference derived from the sperm centrosome. The data here given, as concerns the maturation and fertilization phenomena pertaining to the albino rat, unless otherwise credited, have been drawn from the account of Sobotta and Burckhard, whose account is accompanied by excellent figures.

Long has studied in living ova of mice and rats the phenomena of maturation and fertilization. Tubal ova were placed in Ringier's solution on an especially constructed slide and spermatozoa introduced. It was possible to seminate the ova of rats with rat spermatozoa and to observe the formation of the second polar body. The formation of the second polar body, "usually near the first polar cell, may begin within five minutes to two or more hours after the spermatozoa are introduced. The constriction may be finished three-fourths of an hour later." "The first appearance is an elevation clearer than the rest of the cell. The swelling becomes higher, and at one side of the elevation there appears a depression which is the beginning of the constriction which presently encircles the whole swelling and cuts it off from the egg." Nothing could be said as to the changes which the chromatin undergoes after the spermatozoa have penetrated the egg. The eggs remained alive and apparently normal for about twelve hours, after which they began to degenerate.

PRONUCLEAR STAGE

As has been stated, my own observations on the development of the albino rat (*Mus norvegicus albinus*) begin with the pronuclear stage. The material at hand for this stage is listed in table 1, page 258.

Thus there are present in the series 34 ova showing a pronuclear stage and 9 ova showing the second maturation spindle in the monaster phase. The latter may be dismissed with the brief statement that they represent unfertilized ova. In rat No. 108, with 7 ova in the stage of the second maturation spindle, killed 24 hours after the observed copulation, there was found no trace of spermatozoa in the oviduct. Two reasons may be offered for the non-appearance of fertilization in this case:

TABLE 1

RECORD NUMBER	HOURS AFTER BEGINNING OF INSEMINATION	NUMBER OF OVA	STAGE OF DEVELOPMENT	
			Pronuclear	Second maturation spindle
106	24 hours	8	8	
107	24 hours	11	10	1
108	24 hours	7		7
109	24 hours, 15 min.	9	8	1
110	24 hours, 15 min.	8	8	
Total		43	34	9

Ovulation may have occurred so late that the spermatozoa may have died before the ova reached the ampullar portion of the oviduct. This explanation, it would seem, is invalidated by the fact that the position of the ova in the oviduct, as shown by graphic reconstruction, is essentially the same as in the other four rats studied, and in which fertilized ova were found, so that ovulation must have preceded the killing of the animal by some hours. The other reason, more plausible, attributes non-fertilization to a pathologic condition of the genital tract. In this rat, one ovary was distinctly pathologic, with periovarian capsule greatly distended with a sanguinous liquid, while the upper end of the uterine horn with adjacent oviduct on the other side, as seen in sections, presented evidence of inflammation and epithelial desquamation, in part occluding the lumen. It seemed evident, therefore, that the spermatozoa introduced in the genital tract were unable to penetrate to the oviduct and consummate fertilization. The other two unfertilized ova, found with ova in the pronuclear stage, were in oviducts in which no spermatozoa were found. Both in the mouse and the rat, relatively few spermatozoa reach the upper end of the oviduct; too few, it would seem, to consummate fertilization of all the ova in certain cases. In all of the ova which contained the second maturation spindle, this was in the monaster phase and in tangential position. In size, shape, and chromatin configuration, all presented the characteristics described and figured by Sobotta and Burekhard and Kirkham and Burr, therefore, need not be considered further.

The stage of pronuclei was observed in over 100 ova of the white rat by Sobotta and Burekhard. According to these observers, the two pronuclei show in the earlier stages of their development, large chromatin-like nucleoli, the number of which varies. Some little time later, one or several such chromatoid nucleolar bodies with irregularly formed chromatin masses arranged on the linin network are to be observed. At a still later time, the chromatin becomes distributed over the linin network, throughout the nuclear space, giving the appearance of a fine chromatin network. One of the pronuclei is, as a rule, somewhat smaller than the other. This is regarded as the male

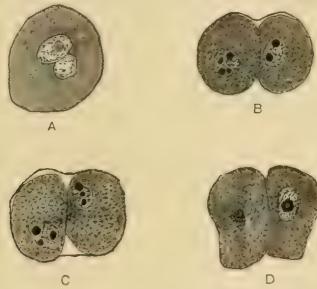


Fig. 1 Tubal ova, albino rat. $\times 200$. A, rat No. 110, 24 hours, 15 min., ovum in pronuclear stage, larger nucleus female pronucleus; B, and C, rat No. 59, 2 days, 2-cell stages, thin oolemma showing in C, only partially seen in B; D, rat No. 62, 2 days, 22 hours, 3-cell stage, the nucleus of the unsegmented blastomere in the monaster phase, only one of the other two cells showing in the figure.

pronucleus, since near it the 'sperm centrum' was now and then observed. The pronuclei lie in about the center of the ovum. The pronuclear stages of my own material, observed in 34 ova, obtained 24 hours after the beginning of insemination—thus at the end of the first day of development—all present essentially the same stage of metamorphosis. As may be seen in A of figure 1, the nuclei are distinctly membraned, and are of relatively large size. The ovum here sketched measures in the stained preparation $70\ \mu$ by $62\ \mu$, and is, therefore, of slightly oval form. Sobotta and Burekhard give $55\ \mu$ to $60\ \mu$ as the size of the tubal ova, and $60\ \mu$ to $65\ \mu$ as the size of the ovarian ova in

the white rat. Kirkham and Burr give the diameter of the living unsegmented egg of the rat as of 0.079 mm. As may be seen from A and B, of figure 2, the tubal ova, even when free in the oviduct, are not of necessity spherical in shape, but often slightly compressed, as may be clearly seen in four models of tubal ova in the pronuclear stage, reconstructed at a magnification of 1000 diameters; in my possession. Depending on the plane of section, the diameter of a tubal ovum may thus vary to the extent of $5\ \mu$ to $8\ \mu$. The two nuclei in the preparation shown in A of figure 1, measure, the larger one, regarded as the female pronucleus, $23\ \mu$ by $16\ \mu$, the smaller $17\ \mu$ by $15\ \mu$. Essentially all of the chromatin is distributed over the linin network in fine granules, the larger nucleus presenting one large, faintly-

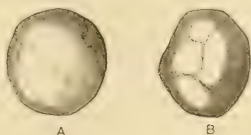


Fig. 2 Models, made after the Born method, of two tubal ova of the albino rat in the pronuclear stage. $\times 200$. A, rat No. 106, 24 hours; B, rat No. 110, 24 hours, 15 min. Reconstructions made at a magnification of 1000 diameters, figure reduced in reproduction.

staining chromatoid nucleolus. The ooplasm is finely granular, distributed so as to give the section a slightly mottled appearance. When compared with figures given by Sobotta and Burekhard (figs. 21 to 24, plates 9-10) showing pronuclear stages of the ova of the rat, my own seem to fall in about the middle of this series, thus some little time after their formation, but not immediately preceding the stage of segmentation spindle formation. In the albino rat, and perhaps in other mammals, the pronuclear stage, in its various phases of nuclear metamorphosis, must constitute a stage covering a relatively long period. If it is assumed that semination occurs about 10 to 12 hours after the beginning of insemination, such assumption being justified by the observations of Sobotta and Burekhard, according to whom the life of the spermatozoa in the genital tract of the white rat is only about 10 hours, and if it is recalled that in

living rat ova Long found that the constriction of the second polar body may be completed three-fourths of an hour after its inception, then it must be evident that the pronuclear stage extends through a period which exceeds 10 to 12 hours, since in none of my pronuclear stages obtained 24 hours after insemination was evidence of first segmentation spindle observed.

In order to determine accurately the relative position of the ova within the oviduct during the pronuclear stage and the stages of segmentation, oviducts containing ova were reconstructed after the Born wax plate method. In form, relations, and general structure, the oviduct of the albino rat is essentially the same as that of the mouse as described by Sobotta. The oviduct of the rat measures from fimbriated end to termination in the uterine horn from 2.5 cm. to about 3.0 cm. It presents eight to ten fairly constant major folds, the middle group of which is closely applied to the ovarian capsule. The upper or distal folds pierce the capsule, ending in the fimbriated end found within the capsule, while the lower or proximal folds, proximal with reference to the uterine horn, effect connection with the uterine horn. These relations are essentially the same as those described by Sobotta for the oviduct of the mouse. This observer recognizes four segments in the oviduct of the mouse, characterized by epithelial lining, nature and extent of folding of the mucosa, and thickness of the musculature. The first segment, which falls to the infundibulum, presents a thin musculature and high mucosal folds with epithelial lining consisting of relatively short cylindrical cells with distinct cuticular border and long cilia. As characteristic of this portion of the tube there are further described accessory nuclei compressed between the epithelial cells. Only this portion of the oviduct is ciliated. In the second segment, the lumen is large and the folds of the mucosa prominent. They are covered by a non-ciliated epithelium, without distinct cuticular border. The musculature is relatively thin. In the third segment the musculature is well developed with circularly and longitudinally disposed cells. The lumen is narrow and the folds are nearly absent, while the epithelium is of a simple columnar variety. The fourth segment, not so well characterized, consists of the loops which make con-

nection with the uterine horns, with folds and epithelium much as in the third segment, and a prominent musculature. In all essentials, this description applies to the oviduct of the albino rat, except that in the first segment the accessory nuclei described by Sobotta as found between the epithelial cells were not evident in the rat. In figure 3, is reproduced a model of a wax reconstruction of the right oviduct of rat No. 106, killed 24 hours after the beginning of insemination, and containing eight ova in the pronuclear stage. This oviduct measured from fimbria to termination in the uterine horn 3.2 cm. It presents 10 major folds, which folds may be recognized with more

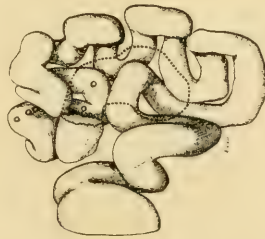


Fig. 3 Model of right oviduct of rat No. 106, 24 hours. $\times 10$. Fimbriated end and infundibulum removed in the drawing so as to expose underlying loops; their relative position given in dotted outline. The position of the ova, which are outlined in circles, is shown as if seen through a transparent wall. The relative position of three of the eight ova found within this tube cannot be revealed in this view of the model.

or less clearness in all the models made and here reproduced. The slight difference in the relative position of these folds as seen in the several figures may be accounted for by the varying degrees of tension to which the tissues were subjected prior to fixation. In rat No. 106, the ovaries with oviduct and upper end of the uterine horn, were excised and placed in the fixing fluid without applying any tension. Of these 10 major folds, the four distal ones, those beginning with the fimbriated end, fall to segments one and two of Sobotta's designation, having a wide lumen and folded mucosa. In the figure, the position of the ova is indicated by small black circles. By reason of the relation of the folds, only five of the eight ova can be brought

to view in the aspect of the model sketched. The position of the first and the last of the series is correctly given. The ova are situated in a loop of the oviduct which is about 8 mm. from the fimbriated end. By the end of the first day after the beginning of insemination, the ova have thus travelled about one-fourth the length of the oviduct. In figure 4 is reproduced a model of a detailed reconstruction of that portion of the oviduct



Fig. 4 Model of the segment of the right oviduct of rat No. 106, 24 hours, containing the ova the general position of which is shown in Figure 3. $\times 50$. The wall is in part removed, so as to expose the lumen. Note the character of the folds of the mucosa. The relative position of the eight contained ova, all in the pronuclear stage, is clearly shown.

containing the ova, representing a loop of the tube with one side cut away, this to show the extent and character of the mucosal folds, the width of the lumen and the relative position of the several ova. The figure presents these facts so clearly that lengthy description is deemed unnecessary. The several ova are distributed through a tube segment measuring about 2.5 mm. in length. They lie free in the lumen, apparently bathed in a fluid from which there is only a small amount of precipitation at the time of fixation. Their position in the oviduct at

this stage, free in the lumen, is well shown in figure 5, which is from a longitudinal section of a loop from the left oviduct of rat No. 109, showing three ova, with but few remaining discus cells and a thread of coagulum linking the ova together, an appearance quite characteristic at this stage. The figure was drawn by aid of camera lucida from a single section. All of the ova, of which there are seven, distributed through this loop, contain two pronuclei; in none of the ova figured do the two pronuclei

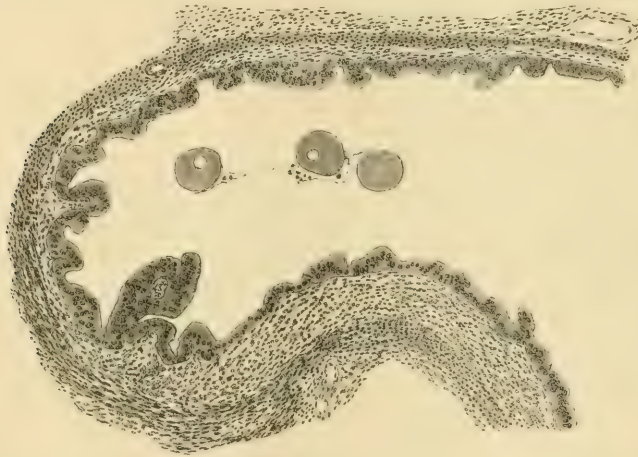


Fig. 5 Camera lucida drawing of a portion of a section of the left oviduct of rat No. 104, 24 hours, 15 min. $\times 100$. Three ova with a few discus cells, are shown as lying free within the lumen. The ova are in the pronuclear stage, not shown in this section, but readily ascertained by tracing through the series. The loop of the oviduct here shown in section is cut longitudinally, thus the folds of the mucosa are not prominent.

fall in the same section. My series contains seven oviducts with pronuclear stages, with accompanying ovary, cut serially. Only one of the oviducts, rat No. 106, was reconstructed in wax. In the other six, graphic reconstructions were made. This permits analysing the loops, determines their sequence, but does not readily admit of measuring their length. In the six oviducts graphically reconstructed, the position of the ova, the number of which varies from one to seven in the several tubes, is essentially as in the wax reconstruction figured. It would appear,

therefore, that in the albino rat, 24 hours after the beginning of insemination, the ova are to be found in the pronuclear stage, with the ova distributed in the end of the third to the beginning of the fourth major loop of the oviduct, a portion of the oviduct having a relatively wide lumen and lined by a much folded mucosa and possessing a relatively thin muscular wall, having thus migrated about one-fourth of the length of the oviduct.

SEGMENTATION STAGES

2-cell stage. The material on which my own observations of this stage are based is listed in table 2.

TABLE 2

RECORD NUMBER	HOURS AFTER BEGINNING OF INSEMINATION	NUMBER OF OVA	STAGE OF DEVELOPMENT
60	1 day, 18 hours	7	2-cell stage
59	2 days	8	2-cell stage
58	2 days, 17 hours	8	{ 7, 2-cell stages; 1, 3-cell stage
61	2 days, 18 hours	8	2-cell stage
62	2 days, 22 hours	11	{ 10, 2-cell stages; 1, 3-cell stage

Thus in all 40 ova after the completion of the first segmentation division and 2 ova in the 3-cell stage, in each of which the undivided blastomere presents a nucleus in mitosis.

My own material lacks stages showing the formation of the first segmentation spindle, the conjugation of the two pronuclei, and the first segmentation division. I am forced to proceed from the pronuclear stage to that showing the first two blastomeres. It was not possible to supplement my material after this was sectioned and the stages determined, since it was only after leaving The Wistar Institute that this gap in my series was recognized. This is the more to be regretted since neither Melissinos, Sobotta and Burekhard, nor Kirkham and Burr, all of whom have considered maturation and fertilization as observed in the albino rat, discuss these stages in their account. In the albino rat, the fusion of the two pronuclei on the first segmentation

spindle, and the first segmentation division would appear to fall to a period ranging from the beginning to near the middle of the second day after the beginning of insemination, probably about 30 to 32 hours after insemination. In the mouse, in which these stages have been very completely and carefully investigated by Sobotta, the conjugation of the pronuclei and the first segmentation spindle formation falls to the end of the first day after copulation. These phenomena appear to be passed through rather quickly in the mouse ovum, covering a period of only about one and a half to two hours.

The 2-cell stage with resting nuclei extends through a relatively long period. In the mouse it extends through nearly an entire day, as shown by Sobotta, who found 2-cell stages present through a period ranging from 25 hours to 48 hours after copulation. Melissinos often observed the 2-cell stage with resting nuclei in both mice and rats in material gathered 24 hours after copulation and to 44 hours thereafter. It is to be regretted that this observer does not differentiate more specifically between ova of mice and rats in his description. As a rule it is impossible to determine except by inference to which of the two varieties of ova his account refers. It may be assumed that the statements made apply equally well to the ova of either the mouse or the rat.

In my own material, the 2-cell stage was observed during a period extending from 1 day, 18 hours to 2 days, 22 hours after the beginning of insemination, thus for a period extending over more than 24 hours. In the albino rat, the first two blastomeres are equivalent cells of essentially the same size and structure, as may be seen from B and C, of figure 1, drawn respectively of ova found in the right and left oviducts of rat No. 59, killed two days after the beginning of insemination, and regarded as representative ova. The two cells of each ovum are not spherical, but of slightly oval form, with relatively large, distinctly membranated nuclei, with fine chromatin granules scattered on the linin network and a number of relatively large chromatoid nucleoli. The cytoplasm presents a granular appearance, the granules being evenly distributed throughout the cell. In my own material, I seldom find the two cells lying in the same plane,

but one cell, as a rule, rises slightly higher than the other. This is more clearly seen in reconstructions than in sections. In figure 6 are shown reconstructions of the 2-cell stages, figured in B and C of figure 1. In B, of the figures, the plane of section is at right angles to the vertical axis of the reconstruction as shown in B of figure 6, while in C of figure 1, the plane of section is parallel to the vertical axis of the reconstruction shown in A of figure 6. The equivalence or non-equivalence of the first two blastomeres of the segmenting mammalian ovum has been the subject of discussion since the time of Van Beneden's fundamental observations on the segmentation of the ovum of the rabbit. This discussion has been summarized a number of times in recent years, and need not be entered into here. Suffice to say that the consensus of opinion of the more recent contributors

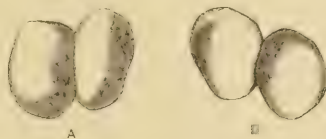


Fig. 6 Models, obtained by reconstruction after the Born method, of the 2-cell stages of the albino rat. Rat No. 59, 2 days. $\times 200$.

is, that the first two blastomeres of the mammalian ovum are equivalent in size and structure if the stage is observed soon after its formation. As above stated, the 2-cell stage of the mammalian ovum extends through a relatively long period, probably about 24 hours. The two cells do not as a rule divide synchronously, the division of one preceding the other by some little time, resulting in a 3-cell stage. The cell to divide first increases slightly in size and presents a clearer protoplasm prior to its division. In a 2-cell stage, viewed in this phase of cytomorphosis, one of the cells appears slightly larger with clearer protoplasm than does the other cell, explaining the difference in size and structure observed by Van Beneden and by other observers who concur in his views. I am convinced that a difference in the size of the two cells may be accounted for by the plane of section in which they are cut, even though the nuclei of both cells are included in the section. In the figures of sections of the 2-cell

stage of the mouse, given by Sobotta and Melissinos, the nuclei of the two cells lie in about their center and essentially in the same plane. In my own material of the 2-cell stage of the albino rat it is not unusual to find the nuclei of the respective cells nearer the opposite poles of the two cells than at their centers, as shown in C, of figure 1. In B of this figure, where the two nuclei appear as lying much nearer the center of the cells, they are in reality placed much as in C, as is shown by the reconstruction.



Fig. 7 Model of the right oviduct of rat No. 59, 2 days. $\times 10$. Not quite the entire oviduct was available for reconstruction, the upper end of the uterine horn thus not shown in the figure. The position of the four 2-cell stages, each of which is outlined in a circle, found within the tube, is shown as if seen through a transparent wall.

To determine the position of the segmented ovum in the 2-cell stage in the oviduct, reconstructions were made of two oviducts. In figure 7 is shown a reconstruction of the right oviduct of rat No. 59, killed two days after the beginning of insemination. In preparing the material for embedding, this oviduct was cut not quite at its insertion into the uterine horn. The portion of the oviduct reconstructed measures 2.29 cm. Nine major loops are shown. The four ova in the 2-cell stage found in this tube are situated in the sixth to the seventh loop at a distance of about 1.4 cm. from the fimbriated end. This portion of the oviduct falls to segment three of Sobotta's designation. It is lined by non-ciliated epithelium resting on a mucosa with inconspicuous secondary folds, but presenting four or five characteristic major folds. This portion of the oviduct is closely

applied to the outside of the ovarian capsule, and conspicuous in all of the figures of models of the oviducts here presented. The detail of the distribution of the ova in the tube is given in figure 8, a reconstruction under a higher magnification of the segment of the oviduct containing the ova. The lumen is exposed so that the character of the mucosal folds may be seen. The ova are spaced in a segment of the tube measuring 3 mm., and are

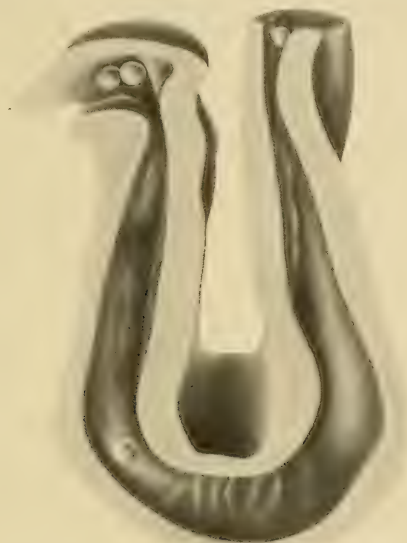


Fig. 8 Model of the segment of the right oviduct of rat No. 59, 2 days, containing the four 2-cell stages as shown in figure 7. $\times 50$. Note the absence of prominent folds in the mucosa. The segment presented in the reconstruction measures 3 mm. The four 2-cell stages contained in this tube are relatively widely spaced.

in this case more widely separated than is usual for this stage. In figure 9, there is reproduced a reconstruction of the left oviduct of rat No. 62, killed 2 days, 22 hours after the beginning of insemination. This tube was also cut a little before its insertion into the uterine horn. The portion reconstructed measures 2.45 cm. In it there are found five ova in the 2-cell stage, situated about 2 cm. from the fimbriated end, and in the last loop of the third segment of the oviduct. The five ova are closely

grouped between two opposing folds of the mucosa. Their general relations are shown in figure 10, a reconstruction under higher magnification of the segment of the oviduct containing

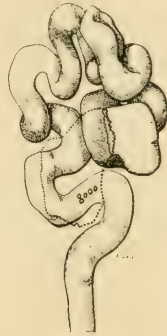


Fig. 9 Model of the left oviduct of rat No. 62, 2 days, 22 hours. $\times 10$. Not quite the entire oviduct was available for reconstruction, thus the relative position of the upper end of the uterine horn is not shown in this figure. Fimbriated end and infundibulum removed in the drawing, so as to expose the underlying loops; their relative position is given in dotted outline. The position of five 2-cell stages, found within this tube, is given as if seen through a transparent wall.

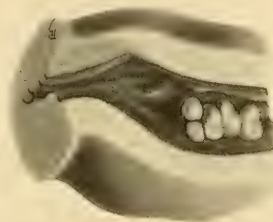


Fig. 10 Model of the segment of the left oviduct of rat No. 62, 2 days, 22 hours, containing the five 2-cell stages, the general position of which is shown in figure 9. $\times 50$. Note the compact grouping of the ova.

the ova, cut so as to expose the lumen. At the magnification used it was not possible to reproduce in the model the exact shape of the several ova, their relative position is, however, correctly given. In all, ten oviducts, containing 40 ova in the 2-cell stage, are included in my series. Of these, two, as above given, were reconstructed by the Born method. The other eight were reconstructed graphically, beginning with the uterine

end of the tubes. In six of these, the ova are quite closely grouped as given in the reconstructions shown in figures 9 and 10. In the remaining two they were more widely spaced, about as shown in figures 7 and 8. In the oviducts taken from rats Nos. 58, 61, 62, killed respectively 2 days, 17 hours, 2 days, 18 hours, and 2 days, 22 hours, after insemination, the ova are found in a portion of the tube which corresponds very closely to that shown in the reconstruction presented in figure 9. In rat No. 60, killed 1 day, 18 hours after insemination, the ova are more widely spaced and are situated in a segment of the oviduct approximately one loop nearer the fimbriated end than that given in figure 7, a model of the oviduct of rat No. 59, killed two days after insemination.

In one of the segmented ova of rat No. 60, the two blastomeres resulting from the first segmentation division are distinctly separated by a space equal to about one-half of the diameter of each of the cells. No oolemma is discernible. The two separated cells appear normal in size, shape, and structure, as do also their nuclei. They lie free in a slightly distended portion of the lumen, and appear not to have been separated as a consequence of manipulation. The possibility of each developing separately is suggested, and may be offered as a possible explanation of the occurrence of very small embryos now and then found among others showing normal development. King states that "On dissecting pregnant females (rats) one frequently finds one or more embryos that are much smaller than the rest. While in some instances such small embryos appear normal and are presumably either runts or embryos that have resulted from superfecundation, in the majority of cases they are pathological, probably because of faulty implantation of the ovum." My own material contains pathologic ova and embryos in different stages of development. This portion of the material will be considered in Part II, where the possibility of the occurrence of half embryos will be discussed.

As may have been seen, the 2-cell stage of the albino rat covers a period of somewhat more than 24 hours, extending from about the middle of the second day until toward the end of the third

day after the beginning of insemination. During this period the segmented ova migrate in the oviduct for a distance equaling nearly half its length. The trustworthiness of the material, it would seem to me, is shown by the fact that in the shorter time stages the segmented ova are situated nearer the fimbriated end, while in the longer time stages they approach the region of the insertion of the oviduct into the uterine horn. This is clearly shown in the reconstructions shown in figures 7 and 8.

A 3-cell stage was observed only twice: in one of eight ova contained in the oviducts of rat No. 58 (2 days, 17 hours) and in one of eleven ova found in the oviducts of rat No. 62 (2 days,

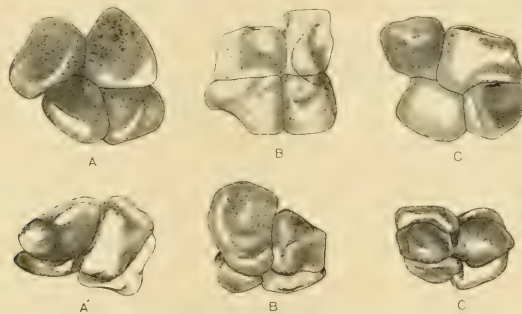


Fig. 11 Two views of each of three models of 4-cell stages of the albino rat. Rat No. 50, 3 days, 1 hour. $\times 200$. A, B, and C, gives a side view, A', B', and C' a vertical view, of each of the three models.

22 hours). All the other ova found in these two animals were in the 2-cell stage. In the two 3-cell stages noted, the undivided blastomeres of each ovum presented a nucleus in mitosis; in one, in the monaster phase, in one, in the diaster phase. The division of the first two blastomeres, resulting in the 4-cell stage, it would appear, occurs in the albino rat toward the end of the third day. The material gathered at the beginning of the fourth day after insemination presents throughout a 4-cell stage. In D of figure 1 is shown reproduced one of the sections of a series of six sections including one of the ova in the 3-cell stage. Only one of the two cells resulting from the division of one of the first two blastomeres is included in the section; the cell in mitosis represents the undivided blastomere.

4-cell stage. The material includes the oviducts of two rats, Nos. 50 and 63, killed 3 days and 1 hour after the beginning of insemination, with twelve ova in the 4-cell stage. In figure 11, there are shown two views of each of the models obtained by reconstruction after the Born method, at a magnification of 1000, of the three 4-cell stages found in the oviducts of rat No. 50. The drawing of the reconstructions do not present the conventional figures of the 4-cell stage of the mammalian egg. In none of the twelve ova of this stage was the plane of section such as to include all of the four cells in one section. Nearly all

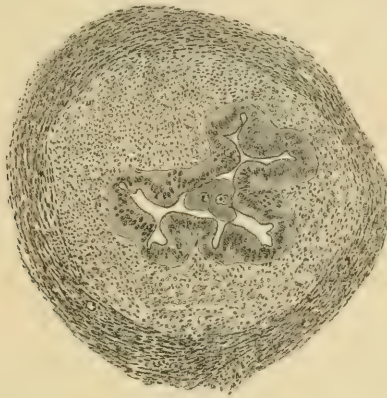


Fig. 12 Cross-section of right oviduct of rat No. 50, 3 days, 1 hour. $\times 100$. This section contains two cells of a 4-cell stage of the albino rat, slightly compressed between the folds of the tubal mucosa.

lie in a portion of the tube which presents a relatively narrow lumen, and appear as if slightly compressed between the folds of the mucosa. I am not disposed to regard this as a resultant of fixation, due to contraction at the time of fixation. In figure 12 is reproduced a cross section of the right oviduct of rat No. 50, passing through a 4-cell stage. It is evident that in shape the two cells included in the section, conform in the main to the form of the lumen, the mucosa appearing as slightly retracted to one side of the egg mass. This conformity in shape of cell mass to the form of the lumen I find quite general in my material showing segmentation stages of the albino rat, to some extent

even in the 2-cell stage, more clearly shown in the 4-cell and later segmentation stages, as will appear from further reconstructions presented. It would seem to me reasonable to assume that these cell masses are of such plasticity that they are molded by the tubal mucosa rather than they would compress the mucosa and maintain an inherent form. A number of segmented ova in presumably the 6- and 8-cell stages were removed from oviducts by injection and studied in warm normal salt solution, in a living state. In the warm normal salt solution the morula masses



Fig. 13 Model of right oviduct of rat No. 50, 3 days, 1 hour. $\times 10$. A short segment of the upper end of the uterine horn, lower part of the figure, is included. The fimbriated end and a part of the infundibulum removed in the drawing so as to expose the underlying loops; their relative position is indicated in dotted outline. The position of the four ova in the 4-cell stage, at the beginning of the last loop of the oviduct, is shown as it seen through a transparent wall.

presented a nearly spherical form, conforming to the conventional illustrations of the same. In none of the sections of fixed material of my series was this the case. The form of the cell mass, assumed by the segmenting mammalian ovum in early stages of segmentation, therefore, seems to me a question more for academic discussion than one of fundamental importance. The right oviduct of rat No. 50 (3 days, 1 hour) was reconstructed after the Born method. This model is reproduced in figure 13, and includes the uppermost end of the uterine horn. The oviduct

measures 2.8 cm. and contains four ova in the 4-cell stage, situated at the beginning of the last loop leading to the uterine horn, 2.25 cm. from the fimbriated end, thus in the fourth segment of the oviduct as of Sobotta's designation. In figure 14 is reproduced a detailed reconstruction of the segment of the oviduct containing the ova, with the convex portion of the wall of this loop, as shown in figure 13, removed. The section reproduced in figure 12, passes through the lower of the three upper ova, shown in reconstruction in figure 14. In the figure of the reconstruction as also in that of the section, is shown the groove in which these three ova lie. The other oviducts con-



Fig. 14 Model of the segment of the right oviduct, rat No. 50, 3 days, 1 hour, containing the four ova in the 4-cell stage, the general position of which is shown in figure 13. The convex portion of the wall of the loop containing the ova is removed, so as to expose the lumen.

taining 4-cell stages were reconstructed graphically, beginning with the uterine end. The position of the ova in each is essentially as given in the model reproduced in figure 13.

8-cell stage. In rat No. 57, killed 3 days, 17 hours after the beginning of insemination, there are found in the left oviduct, six segmented ova in the 8-cell stage and one segmented ovum in the 11-cell stage. The right ovary and oviduct was injured in the process of embedding and could not be used for sectioning. The ova are spaced in the loop of the oviduct which terminates in the uterine horn. Six of the segmented ova were reconstructed, the seventh was not detected at the time the reconstructions were made. The six models obtained are reproduced

in figure 15, two views of each model being shown. Five of the models, A to E, show 8-cell stages. In F, there is figured an 11-cell stage, three of the cells having completed the next following division. As may be seen from the figures, the form of these morula masses is not spherical but in the main slightly oval, with further irregularities better shown in the models than in the illustrations, due to the fact that the egg masses conform to the shape of the lumen of the oviduct in the region in which they are found. The mucosa lining the segment of this

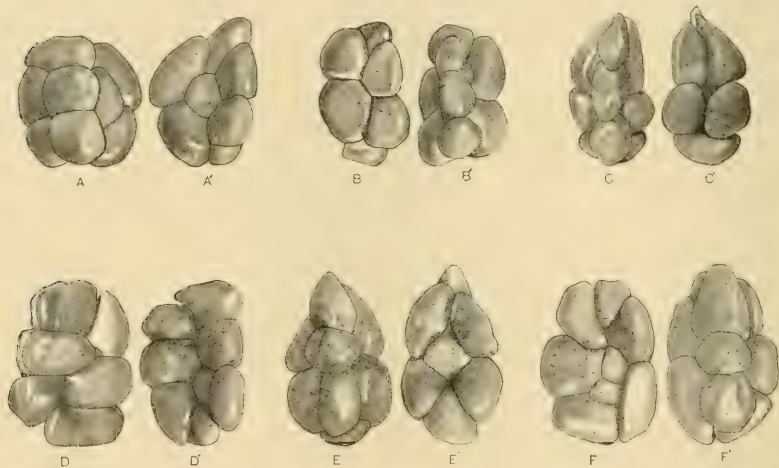


Fig. 15 Models, obtained by reconstruction after the Born method, of 8-cell and 11-cell stages of the albino rat. Rat No. 57, 3 days, 17 hours. $\times 200$. Two views of each model is presented. A-A', to E, E' are of models of 8-cell stages; F and F' of a model of a 11-cell stage.

oviduct containing the ova presents four quite regular longitudinal folds. In figure 16, there is presented a model of a detailed reconstruction of the segment of the oviduct containing the ova, their relative position in the tube and their relation to the major folds is clearly shown. One of these folds it was necessary to in part remove so as to bring to view in the drawing certain of the ova. In figure 17, there is reproduced a portion of one of the sections of the series from which the model shown in figure 16 was made. The fold of the mucosa occupying the center



Fig. 16 Model of the segment of the oviduct, rat No. 57, 3 days, 17 hours, containing the ova shown in fig. 15. $\times 50$. A portion of the wall of the oviduct and a part of the major folds of the mucosa are removed in the drawing so as to expose the contained ova. The relative position of the seven ova found in the tube is shown, as also the extent and character of the folds of the mucosa. The exact form of each of the several ova could not be reproduced in the model at the magnification used; their position is given correctly.



Fig. 17 Camera lucida drawing of a portion of a section of the left oviduct of rat No. 57, 3 days, 17 hours. $\times 100$. This section is of the series of sections from which the models shown in figures 15 and 16 were made. Sections of four 8-cell stages, as seen in a single section, are included. The close proximity of three of these ova, their relation to the tubal wall and mucosal folds is to be noted.

of the drawing, and greatly occluding the lumen, is the fold removed in the model. In this very fortunate section four of the morula masses are included; all are of the 8-cell stage and represent in section the four ova which are placed closely together as seen in the model figured in figure 16. In figure 19, A, there is reproduced at higher magnification another of the sections of the series, including the right one of the three ova in close apposi-

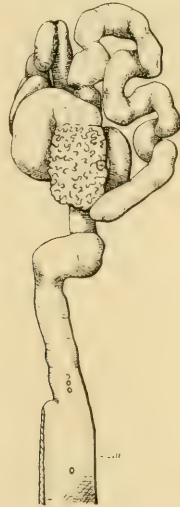


Fig. 18 Model of the left oviduct of rat No. 51, 4 days. $\times 10$. A short segment of the upper end of the uterine horn was included in the reconstruction, lower end of the figure. The position of three of the morula masses, 12-cell to 16-cell stages, in the terminal part of the oviduct is to be noted, a further one is located in the upper part of the uterine horn. These are shown as if seen through a transparent wall. A fifth morula, situated in the uterine horn about 1.5 cm. from the entrance of the oviduct, is not included in the figure.

tion as seen in figure 17, showing six of the eight cells, each cut in the plane of its nucleus. In both of these figures (figs. 17 and 19) the morula masses, as seen in the sections drawn, present a quite regular oval outline. In succeeding sections, in which the mucosal fold and the wall of the oviduct approximate, the cross diameter of each of the four morula masses becomes greatly reduced, they appearing in the final sections of the series in which they are included as narrow, non-nucleated bands of protoplasm.

This series, it seems to me, corroborates the statement previously made, that the detail of form of the living segmenting ova of certain mammals, while in transit through the oviduct, is in a great measure dependent on the configuration presented by the lumen of the oviduct in the particular region in which they are found.

12-cell to 16-cell stages. Rat No. 51, killed 4 days after the beginning of insemination, presents the end of the segmentation stages in the oviduct. In the genital tract of this animal there were found eight morula masses, five on the left side and three on

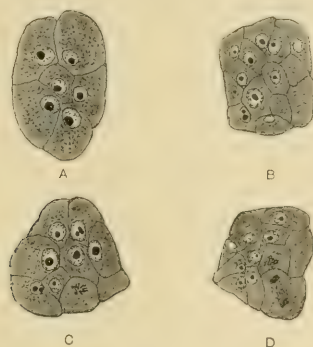


Fig. 19 Sections of morula stages of the albino rat. $\times 200$. A, 8-cell stage, rat No. 57, 3 days, 17 hours; six of the eight cells, each cut in the plane of its nucleus, are included in the section figured. B, C, and D, 12-cell to 16-cell stages, from right oviduct, rat No. 51, 4 days.

the right side. It is somewhat difficult to determine definitely the number of cells constituting each of the morula. The number appears to vary between 12 and 18, though nearly all of the morula masses show certain nuclei in mitosis. The left oviduct with a short adjoining segment of the uterine horn was reconstructed. Slight tension was applied to the tissue prior to fixation, which accounts for the elongation of the proximal loop of the oviduct. The model is reproduced in figure 18. As is evident on study of this figure, three of the morula masses are situated in a portion of the oviduct just prior to its insertion in the uterine tube. These are closely grouped between folds of the mucosa. A fourth morula is found in the uppermost part of the uterine

cavity, just distal to the opening of the oviduct, lying free in a slightly distended portion of the lumen. This morula is of irregular discoidal form, presenting an appearance which suggests that it was fixed soon after it escaped from the oviduct. A fifth morula, of regular oval form, comprising very probably 18 cells, all of which present resting nuclei, is lodged in a shallow pit of the uterine mucosa a little over 1 cm. from the tubal opening. This portion of the uterine horn was not included in the reconstruction, the position of this morula is not, therefore, indicated in the figure. It is evident that this tube was fixed while the several morula masses were in transit from the oviduct to the uterine horn, which occurs, to judge from the material at my disposal, at the end of the fourth day after the beginning of insemination. The morula masses of the right tube are situated in the oviduct just before its point of insertion into the uterine horn, in about the same relative position as are the three upper morula masses of the left side, as shown in the reconstruction. They are of discoidal form, in close relation and appear to comprise, the one 12, the other two 14 to 16 cells. In B, C and D of figure 19 are reproduced sections of each of these three morula stages. The figures, however, are delusive in that the section for each passes through the greatest diameters of the respective morula.

The material at hand permits the conclusion that in the albino rat the segmenting ova pass from the oviduct to the uterine horn at the end of the fourth day after the beginning of insemination, probably in the 12-cell to 16-cell stages. With the beginning of the fifth day, as will appear from further discussion, all of the ova are to be found in the uterine horn.

SUMMARY OF SEGMENTATION STAGES, RATE, AND VOLUME CHANGES

The following summary of the data (table 3) gained by a study of the models of oviducts containing ova in stages from the pronuclear to 12-cell to 16-cell stages in which latter stage transit to the uterine horn occurs, is presented to indicate rate of transit within the oviduct. The regularity of the rate of transit

as revealed in the summary may perhaps speak for the trustworthiness of the age data as concerns my material.

It will be observed that the ova approach the uterine end of the oviduct while in the 2-cell stage; transit through the last portion of the oviduct, where the greater part of the segmentation occurs, being relatively slow. It is hoped that these data, for the accuracy of which I am dependent on reconstructions, may be of service to others who may desire to collect segmentation stages of the albino rat.

TABLE 3

RECORD NUMBER	SIDE RECONSTRUCTED	AGE	NUMBER OF OVA	STAGE	LENGTH OF OVIDUCT	DISTANCE OF OVA FROM FIMBRIA	RELATIVE LENGTH OF TUBE TRAVERSED
					<i>cm.</i>	<i>cm.</i>	
106	R	1 day	8	pronuclear	3.2	0.8	0.25
59	R	2 days	4	2-cell	2.29*	1.4	0.61
62	L	2 days, 22 hrs.	5	2-cell	2.45*	2.0	0.82
50	R	3 days, 1 hr.	4	4-cell	2.8	2.5	0.90
51	L	4 days	5	12- to 16-cell	2.86	2.86	1.00

*Not the entire length of oviduct was available for reconstruction.

In order to obtain the volume changes of the ova during transit through the oviduct, beginning with the pronuclear to 8-cell to 11-cell stages, the following procedure was adopted. As has been shown by my figures, reconstructions were made at a magnification of 1000 diameters of ova presenting the stage in question. The sections of my series measure 10 μ in thickness. In order, therefore, to obtain the correct third dimension, it was necessary to use wax plates 10 mm. thick, in actual practice, five superimposed 2 mm. plates. For the majority of the sections of my series this procedure was relatively simple. However, there was usually a question as to the thickness to be ascribed to the first and last section of any given series, since it was evident, both from the appearance of the section, as seen under the magnification used, and the appearance of the model, that the end sections did not measure 10 μ in thickness, and it

was necessary to reduce proportionately the thickness of the wax plate representing them. As a rule, these were made about one-half the thickness of the other plates. The irregularities revealed by the rough model after superimposing the respective plates, not so marked as might be supposed considering the thickness of the plates used, were adjusted, not by trimming the model and cutting away wax, but by smoothing with warm irons. The possibility of error is admitted, but since all of the models were made in the same way, errors if committed were probably essentially the same for all of the models. The volumes of the models were obtained by weighing the water displaced by each, and after making the necessary temperature corrections, reducing weight of water displaced to volume. The average of several determinations is given in table 4.

TABLE 4

RECORD NUMBER	AGE	STAGE	ACTUAL VOL. OF EGG MASS IN C. MM.	AVERAGE VOL. PER STAGE GIVEN IN C. MM.
106	1 day	pronuclear	0.00015058	0.000155693
106	1 day	pronuclear	0.00014317	
106	1 day	pronuclear	0.00015775	
106	1 day	pronuclear	0.00017127	
59	2 days	2-cell stage	0.00016240	0.000172565
59	2 days	2-cell stage	0.00018273	
50	3 days, 1 hr.	4-cell stage	0.00018338	0.000162443
50	3 days, 1 hr.	4-cell stage	0.00015520	
57	3 days, 17 hrs.	8-cell stage	0.00018893	
57	3 days, 17 hrs.	8-cell stage	0.00016040	
57	3 days, 17 hrs.	8-cell stage	0.00018653	0.000183516
57	3 days, 17 hrs.	8-cell stage	0.00018193	
57	3 days, 17 hrs.	8-cell stage	0.00019979	
57	3 days, 17 hrs.	11-cell stage	0.00021025	

The uniformity of the figures giving the actual volume of the egg mass, as determined by the weight of the water displaced by the models of the respective ova reconstructed, leads me to feel that the errors committed in reconstruction were not serious. The last column of the table, giving averages, is of interest since it shows a very slight increase in the volume of the egg mass during segmentation and transit through the oviduct. Following the pronuclear stage, which, as has been seen, ex-

tends through a relatively long period and into the beginning of the second day, by which time the ova have migrated about one-fourth of the length of the oviduct, there occur only three successive mitotic divisions, including the first segmentation division, namely mitoses resulting in 2-cell, 4-cell and 8-cell stages while the ova are in transit in the oviduct. In making this statement it is assumed that in the successive segmentations, the several cells divide synchronously, which is not in conformity with the fact. These three mitotic divisions are spaced at intervals of about 18 hours. In the next following division, the fourth, the ovum passes from the oviduct to the uterine horn. Since the normal gestation period of the non-lactating albino rat is only 21 to 23 days, this slow rate of increase in volume and multiplication of cells during the first four days of development is of especial interest and is very probably to be accounted for by the inadequacy of the food supply of the ovum during its transit through the oviduct.

The presence or absence of the oolemma has not been considered in discussing the segmentation stages of the albino rat. In my own material, the oolemma was clearly observed in certain of the 2-cell stages, but not in the 4-cell nor 8-cell stages. Widakowich reports that he has observed in the albino rat, loss of the oolemma even in the 2-cell stage. Since all of the material covering these stages was fixed in Carnoy's fluid, a fluid with a relatively large glacial acetic acid content, it may be questioned as to whether the fixative used may not be in part responsible for the early disappearance of the oolemma, though neither Hubrecht nor Sobotta considers the presence or absence of an acid in the conserving fluid of special moment in the fixation of the oolemma. Sobotta finds that the oolemma disappears in the ova of mice during the 8-cell stage. The early disappearance of the oolemma in the albino rat may be offered as an explanation of the fact that the egg mass during segmentation and transit through the oviduct does not, as a rule, present a spherical form but appears compressed and molded to fit the form of the lumen. A similar explanation is offered by Sobotta to account for the irregularity of form assumed by the ovum of the mouse after loss of the oolemma. In the forms in which the oolemma

persists through the later stages of segmentation, as for instance in the rabbit, the morula mass presents a spherical form. The transit of the ova through the oviducts is effected, very probably, through peristaltic action of the muscular coat, since only a relatively short portion is lined by ciliated epithelium. Whether or not there exists a rhythmic periodicity in the peristaltic action, it is impossible to state. The fairly regular rate of transit argues for the presence of some regulatory mechanism. The compact grouping often presented by a series of ova in transit through the oviduct, especially after reaching the portion with narrower lumen, suggests peristaltic action.

The literature dealing with the segmentation stages of the albino rat is very meagre. Grosser figures what is presumably an 8-cell stage. His figure 27 is referred to only incidentally in the text, but in the accompanying legend it is stated that the figure shows "three ova of the white rat in process of segmentation, with zona pellucida, in transit through oviduct, three and one-half days after insemination." If I am right in interpreting these ova as in the 8-cell stage, this corresponds very closely to my own observation on rat No. 57, 3 days, 17 hours (figs. 15-17). It is impossible to draw definite conclusions as to the segmentation of the ova of rats from the account of Melissinos. This observer while he states that his material includes the ova of mice and rats, and while considering segmentation mentions the ova of both forms, discusses them without differentiating between the two. His figures all refer to ova of the mouse. Selenka, Robinson, and Widakowich, who have contributed to our knowledge of the embryology of the albino rat, do not include the segmentation stages, to be found in the oviduct, in their account.

The rate of segmentation and the time of transit through the oviduct, as given in the literature for certain other mammals is as follows: Sobotta has shown for the mouse that the 2-cell stage is reached about 24 hours after copulation, the ovum remaining in this stage to about the 48th hour. The 4-cell stage was observed at about 50 hours, the 8-cell stage at 60 hours, and the 16-cell stage at 72 hours 'post coitum.' The ova of the mouse pass into the uterine horn about 80 hours post coitum,

thus the beginning of the fourth day, in a stage in which 16 cells up to 32 cells may be enumerated; the oolemma having been lost in the 8-cell stage. The data furnished by Melissinos as concerns the mouse, are as follows: The 2-cell stage is obtained at the end of 24 hours after copulation, the 6-cell stage during the first 12 hours of the second day, and the 28-cell stage during the second 12 hours of the second day. The ovum is said to pass into the uterine horn at the end of the third day after copulation, retaining its oolemma. The account of Sobotta seems the more reliable. Hensen describes a 2-cell stage in the guinea-pig 22 to 24 hours after copulation, and Bischoff records that the ovum of the guinea-pig passes into the uterine horn while in the 8-cell to 16-cell stage, toward the end of the third day. Heape, who has described very fully the segmentation stages of the mole (*Talpa europea*) gives no data as to the rate of segmentation. In the explanation of the figures presented it may be noted that the ova figured, showing 2-cell to 15-cell stages, were taken from the oviduct. His figure 20, showing an ovum 'fully segmented' was obtained from the anterior end of the uterus. Assheton gives for the rabbit the following data: The 2-cell stage is obtained about 24 hours and the 4-cell stage about 26 hours after coitus. The third series of divisions begins about 28 hours after coitus, so that by the end of the second day a typical morula of 16 cells to 20 cells is to be found. Between 73 hours and 96 hours the beginning of the blastodermic vesicle formation is to be noted. Ova obtained 80 hours after coitus, still surrounded by the oolemma, were removed from the uterine horn. Data as to the relative position of the ova in the oviduct in the several stages of development discussed, are given. As concerns the sheep, Assheton states that the ova pass into the uterine horn early on the third day after mating. The pronuclear stage is to be observed the second day, and the first segmentation at the end of the second day. By the fourth day, with the ova in the 8-cell stage, they are found in the upper end of the uterine horn. The blastodermic vesicle formation begins with the 16-cell stage. Again, according to Assheton, the ova of the pig pass to the uterus about the third day after fertilization, if I read him rightly, reaching the uterus in the 4-cell stage, although ova in the 2-cell

and 3-cell stages were obtained from the upper end of the uterine horn. The presence of 2-cell stages in the uterine horn has also been noted by Keibel, in *Erinaceus europaeus*, by Van Beneden in the bat, and by Hubrecht in the insectivor *Tupaya javanica*. Finally, it may be noted that according to the observations of Bischoff, the segmenting ovum of the dog occupies 8 to 10 days after insemination in transit through the oviduct.

COMPLETION OF SEGMENTATION AND BLASTODERMIC VESICLE FORMATION

The material covering the end stages of segmentation and the early stages of blastodermic vesicle formation is listed in table 5.

TABLE 5

RECORD NUMBER	AGE	NUM- BER OF OVA	STAGE
64	4 days, 14 hrs.	5	Early stage of blastodermic vesicle formation
52	4 days, 15 hrs.	8	Morula, beginning of segmentation cavity, early stage of blastodermic vesicle
55	4 days, 16 hrs.	1	Early stage of blastodermic vesicle
68	4 days, 16 hrs.	4	Early stage of blastodermic vesicle
53	5 days	7	Early stage of blastodermic vesicle
56	5 days	5	Early stage of blastodermic vesicle

Thus there are at hand 30 ova, showing late morula stages, the beginning of segmentation cavity formation and early stages of the blastodermic vesicle, falling in the latter half of the fifth day after the beginning of insemination. In all of the uteri from which this material was taken, the ova are spaced in the uterine horns about as in later stages of development; they lie free in the uterine lumen, are in the main ovoid in form, their long axis presenting no definite relation to the long axis of the uterine horn. In preparing this material for sectioning, it was the custom to cut an entire uterine horn into segments measuring about 1.0 cm. to 1.5 cm. in length. These segments were then embedded so as to admit cutting longitudinally and in a plane parallel to the plane of the mesometrium. Cut in this way, the majority of the ova were cut longitudinally or nearly so, others in an oblique plane, others again, crosswise. Since it

is impossible to orient the ova prior to sectioning, the securing of desirable sections is a matter of chance. The difficulty is further enhanced by reason of the fact that owing to shrinkage as a result of the action of the fixing fluid, the ova in the vesicle stage are apt to be more or less folded, so that even though the plane of section may be that desired, the resultant sections lose in value by reason of this folding.

It has been shown that in the albino rat, the ova pass from the oviduct to the uterine horn toward the end of the fourth day. During the first half of the fifth day, the migration of the ova from the oviduct to the uterine horn appears to be completed, so that by the second half of the fifth day the ova are spaced in the uterine horn about as after fixation to the uterine mucosa. As to the factor or factors which play a rôle in the descent of the ova through the uterine horn and their fairly regular spacing, my own material gives no data; these changes occurring, apparently, during the first half of the fifth day, covering which my material is lacking. Widakowich, who has given especial study to these questions, presents the following considerations: In the downward migration of the ova in the uterine horn, it cannot be assumed that the ova are capable of active movement nor can their motion be ascribed to the action of gravity. While peristaltic action may play a part, it is difficult to see how peristalsis could be so regulated as to space the ova fairly regularly within the uterine cavity. The presence of a ciliated epithelium in the human uterine cavity during the intermenstrual period suggested the presence of a ciliated epithelium in the uterine horn of the rat. After many preparations had been searched in vain for its presence, Widakowich found short cilia, not more than $2\ \mu$ long in the epithelium lining the uterine cavity of a rat killed four days after copulation, and containing ova in the blastodermic vesicle stage. It would appear, therefore, that the uterine epithelium of the rat presents a ciliary border for only a relatively short time, and that the transportation of the ova within the uterus is effected by the cilia. Mandl also found, his material however not including the rat, that cilia are present in many animals on the epithelium lining the uterus only at certain periods, and perhaps only relatively

short periods. While the presence of cilia may explain the migration of the ova in the uterine tube, Widakowich can offer no conclusions as concerns the regulatory mechanism by means of which the ova are spaced at fairly regular intervals in the lumen of the uterus. In none of my sections of the uteri of albino rats, obtained during the fifth day after insemination, have I been able to note the presence of cilia on the uterine epithelium, even when sections were studied under the oil immersion. After reading the account of Widakowich, their presence was looked for in all pertinent stages, but without success. Especially in rat No. 50, in which the ova were passing from the oviduct to the uterine horn was careful search made, but nothing like a distinct ciliary border, composed even of short cilia, was ascertained. In the left genital tract of this rat, as has been stated, three ova were found in the terminal part of the uterine end of the oviduct, one in the uterine lumen just distal to the mouth of the oviduct, and one a little over a centimeter from this opening. The latter was lodged in a shallow depression of the uterine mucosa, as is characteristic for stages lying free in the lumen. The question as to whether this ovum was permanently lodged is difficult to answer. If this is assumed, it is further necessary to assume that the other ova would need to pass it to reach the more distal parts of the uterine lumen.

The literature contains no definite statements as concerns the reactions of the epithelium and mucosa of the uterus to the ova soon after their appearance in the uterine cavity. Widakowich summarizes the views by stating that "It is generally stated, that so long as the ova lie free, the uterus shows no changes." He himself notes that at this time the mucosa presents evidence of marked new formation of capillaries. Burekhard, who had at his disposal a large number of stages showing implantation of the ovum of the mouse, discusses at length the appearance presented by the uterus soon after the ova enter the same and the lodgment of the ova therein. This observer notes that in the non-gravid uterus of the mouse, the lumen lies more or less eccentric, and towards the mesometrial border.

The lumen is not smooth, but presents numerous radially arranged folds, certain of which are relatively deep. Essentially the same characteristics pertain to the mucosa of the uterus, soon after the beginning of gravidity. As the ova pass from the oviduct to the lumen of the uterus they become lodged in certain of the mucosal folds, and generally in certain of the deeper ones to be found along the anti-mesometrial border. I find Burekhard's account of the form of the lumen of the uterine horn, of the structure of the mucosa in early stages of gravidity, and the lodgment of the ova, pertaining to the mouse, applies equally well to the albino rat. No reason can as yet be given as to why the ova are lodged in the mucosal folds in which they are found, and not in others. So far as may be ascertained from the sections, the particular mucosal folds in which the ova are found, do not differ in form and structure from neighboring folds. It is possible that by reconstruction of the epithelial lining of the entire uterine horn in pertinent stages, certain characteristics of form and position might be revealed as possessed by certain mucosal folds which make them especially favorable for the lodgment of the descending ova. Such reconstructions, however, have not been made. Burekhard states that in the mouse, about the middle of the fifth day, after the ova have been in the uterine cavity for a number of hours, there may be observed the first changes in the uterine wall. The changes consist primarily in a flattening of the uterine epithelium. In the immediate region where implantation is to occur, the lining epithelial cells present instead of a cylindric form, a cubic form. The area is sharply demarked from the surrounding epithelium, the transition of cubic to cylindric epithelium being marked by a sharp-lipped epithelial ledge. In my own material of the rat covering these stages, the uterine mucosa likewise presents shallow pits, in the immediate regions where the ova are lodged, lined by slightly flattened, cubic epithelium, very much as described by Burekhard for the mouse. Widakowich presents an excellent figure (fig. 2 of his contribution, rat four days after fertilization—'Befruchtung') showing clearly the relations of the ova to the uterine wall. In this rat, the

uterine epithelium presented a ciliary border, present even in the shallow pit lodging the ovum sketched. He argues from this that the shallow depression and the flattening of the epithelium are not a result of pressure exerted by the vesicle, as thought by Sobotta and Melissinos, but must be due to an active change in the epithelium itself. The mucosa underlying the shallow pits presents at this stage no change of structure. I am thus in accord with Widakowich when he states that he was not able to observe in the mucosa of the rat in the early stages of gravidity, the giant cells described by Disse as found in the uterine mucosa of *Arvicola arvilis*, in similar stages.

The form presented by the ova of the albino rat, in the late morula stages and the early stages of blastodermic vesicle, is ovoid, as may be seen from the figures to be presented. Widakowich is inclined to believe that the form of the blastodermic vesicle of the rat is in a measure dependent on the general form of the space in which it is lodged. He figures two vesicles (figs. 1-2) one of which is nearly spherical, the other of distinctly oval form. Duval (figs. 73-83) presents vesicles having ovoid, triangular, and spherical forms. Christiani's figures covering these stages, are too schematic to be of any value in drawing conclusions. I fear Robinson's account is based on imperfectly fixed material. He states that "toward the end of the fifth day, or the commencement of the sixth day, the longitudinal axis of the blastodermic vesicle is $125\ \mu$ long. During the sixth day, that axis is diminished, first to $95\ \mu$, and then to $64\ \mu$, after which it again increases, and at the commencement of the seventh day, it is $121\ \mu$." Neither Fraser nor Selenka describes nor figures the stages here considered. In the mouse, according to the accounts of Melissinos, Burckhard, and Sobotta, the form of the blastodermic vesicle in early stages is spherical.

The more specific consideration of my own material I shall introduce with a discussion of three stages taken from the uterus of rat No. 52, killed 4 days, 15 hours after the beginning of insemination. In A, of figure 20, there is reproduced the middle one of seven sections of a late morula stage. This morula is of ovoid form, measuring $85\ \mu$ in its long diameter, $54\ \mu$ in its

broad diameter—that is, in plane of sections, and since it passes through seven sections of 10 thickness, measures approximately $70\ \mu$ in its third dimension. It consists of 24 cells, as estimated by counting the nuclei contained in its several sections. The cells vary in size as well as in shape. The nuclei are for the main of spherical form, presenting one or several large nucleoli and fine chromatin granules. This morula is found within a fold of the mucosa, each side of which presents a slight depression, lined by slightly flattened epithelium. This morula mass lies



Fig. 20 Sections of morula mass and early stages of blastodermic vesicle of the albino rat. $\times 200$. A, B, C, rat No. 52, 4 days and 15 hours. D and E, rat No. 68, 4 days and 16 hours. A, late stage of morula; B, shows the very beginning of the formation of the segmentation cavity; C, D, E, early stages of blastodermic vesicle, in E, a distinct covering layer in the thicker portion or floor of the vesicle is evident.

free in the lumen of the mucosal fold, and not in contact with the uterine epithelium. The outline and extent of the shallow depressions found in the opposing walls of the mucosal folds conform to shape and size of the morula mass contained, which appear as if slightly retracted as a result of fixation.

In B, figure 20, is figured one of the sections of a series passing through a morula stage, comprising as estimated 30 cells and measuring $90\ \mu$, by $60\ \mu$, by approximately $50\ \mu$, in which the very beginning of the formation of the segmentation cavity is shown. Near one pole the outermost cells have separated slightly from the more deeply placed cells, so that an irregularly shaped

cavity, eccentrically placed and passing clearly through two of a series of five sections of $10\ \mu$ thickness, is evident. The small cleftlike cavity is bounded by the surrounding cells, the outline of which is distinct. So far as may be judged from the appearance noted as presented in the two sections in which this cavity is found, this arose as a single space and as a result of the separation of the enclosing cells.

In C, of figure 20, there is presented a slightly older stage showing the blastodermic vesicle formation and measuring $80\ \mu$, by $50\ \mu$, by approximately $50\ \mu$; comprising as is estimated, 34 to 36 cells. Unfortunately, the lower part of this vesicle is slightly folded as is shown in the lower left of the figure. The appearances presented in the sections are reproduced as faithfully as could be. Owing to the folding, a portion of the thin wall is cut tangentially. The more darkly colored curved line represents in reality the outer boundary of this portion of the vesicle. The segmentation cavity in this vesicle is distinctly larger than that shown in B of this figure. In the section reproduced the segmentation cavity is bounded for the greater part by four somewhat flattened cells, the increase in the size of the cavity being accompanied, it would seem, by a flattening of the enclosing cells.

In these three closely approximated stages, which, since they are taken from the same uterus are probably separated in time of development by only short intervals, the cells though varying in size and shape, show no essential or fundamental difference in structure, neither in cytoplasm nor nuclei; nor do they show any regularity in arrangement. Only few mitotic figures are to be observed; none in the morula mass shown in A, and but two in each of the other two stages, shown in B and C. Judging from these preparations, one would be led to conclude that segmentation cavity formation in the albino rat is not associated nor accompanied by active cell proliferation. This point will be referred to again after the presentation of further material at hand. In slightly older stages of the blastodermic vesicle than here considered, the thicker portion of the vesicle is designated by Sobotta and others as its floor, which is directed

toward the mesometrial border of the uterine horn, while its thinner portion is designated as its roof, directed toward the antimesometrial border. Therefore, in slightly older stages than thus far figured, the vesicle lies with its long axis approximately at right angle to the long axis of the uterine horn. In further description of the blastodermic vesicle, I shall use the term 'floor' and 'roof' as here specified. In D and E of figure 20, there are reproduced typical sections of the two blastodermic vesicles taken from the uterus of rat No. 68, killed 4 days, 16 hours after insemination. Vesicle D measures $90\ \mu$ by $30\ \mu$ by approximately $60\ \mu$, and is of distinctly ovoid form and slightly compressed. This vesicle is found lying free in a long but narrow fold of the mucosa, both sides of which are slightly molded in conformity with the form of the vesicle. The long axis of the vesicle is still parallel to the long axis of the uterine horn. The roof of the vesicle appears as if slightly contracted, though when traced through the series of six sections it does not appear folded. The roof is composed of only a few cells, perhaps seven in all. The segmentation cavity presents a regular outline. This vesicle supports the contention of Widakowich, that the form of the blastodermic vesicle of the rat is dependent in a measure on the form of the space in which it is found. Vesicle E, of figure 20, measuring $85\ \mu$ by $45\ \mu$ by approximately $40\ \mu$, presents a roof that is slightly folded and shows an early stage in segmentation cavity formation. A figure of the vesicle is included since it represents more clearly than any other blastodermic vesicle of the albino rat in my possession, a differentiation of a layer of surface cells in the mass constituting its floor. This is a question to be more fully considered in further discussion.

In all the measurements of blastodermic vesicles thus far given, even in those given for the morula mass shown in A, figure 20, it is evident that one of the short diameters is appreciably shorter than the other. The vesicles are not only of ovoid form, but slightly flattened, so that even when not folded, the form of the vesicle as seen in section, even when cut parallel to the long axis of the respective vesicles, is dependent in a measure on the plane of the section, whether parallel to the longer or the shorter

of the two cross diameters. This may be seen from the series of drawings made of a blastodermic vesicle cut cross-wise, taken from the uterus of rat No. 68, from which were also taken the two vesicles shown in D and E of figure 20. This series of figures is shown in figure 21, in which are reproduced in serial order the seven successive cross sections into which the vesicle was cut. It measures $65\ \mu$ by $38\ \mu$ by approximately $70\ \mu$, and is found at the bottom of a mucosal fold, found at the mesometrial border, and is resting with one side on the epithelial lining of a shallow pit, the other wall of this mucosal fold, also showing a shallow pit, is slightly retracted. From a study of this series



Fig. 21 A complete series of cross-sections of an early stage of blastodermic vesicle of the albino rat. $\times 200$. Rat No. 68, 4 days and 16 hours. A to C, sections through roof of vesicle, showing segmentation cavity; D to G, sections through floor of vesicle.

of sections, I feel certain that the plane of section is cross and not oblique to the long axis of the vesicle. The roof of this vesicle passes through three sections, A, B and C. The segmentation cavity has thus a depth of less than $30\ \mu$. The overlapping of the cells surrounding the segmentation cavity is to be noted, especially as seen in B of this figure. This arrangement of the cells may explain how the cavity may be enlarged without a material increase in the number of the enclosing cells—in part, by a flattening out of the cells, in part by a rearrangement of the relations of the cells. In the figures of the sections passing through the floor of this vesicle, D to G, attention is drawn to the size, form and relations of the cells and to the fact that there is no distinct covering layer. In this series of sections, there are

shown in all 35 nuclei, two of which are in a late diaster phase. By excluding the nuclei that appear to be cut in two, appearing thus in two successive sections, I estimate that the blastodermic vesicle is made up of only about 30 to 32 cells.

In figure 22, there are reproduced typical sections of four blastodermic vesicles taken from the uterus of rat No. 53, killed five days after the beginning of insemination. This uterus contained in all, eight vesicles, one of which was distinctly pathologic. The four vesicles selected for reproduction and discussion present each certain characteristics worthy of consideration. Vesicle A, which presents an early stage of segmentation cavity forma-

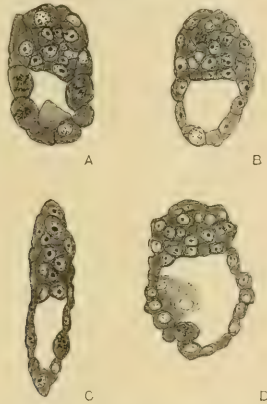


Fig. 22 Sections of early stages of blastodermic vesicle of the albino rat, $\times 200$. Rat No. 53, 5 days.

tion is of interest owing to the number of mitotic divisions it contains. As a rule, I have noticed only a few mitoses at this stage. In this vesicle, which measures $90\ \mu$ by $55\ \mu$ by approximately $40\ \mu$, there are no less than five mitoses to be noted, three of which are in cells forming the roof of the small segmentation cavity, and are included in the section figured. This is the only vesicle in my possession in which an increase in the size of the segmentation cavity is accompanied by active mitoses in the cells bounding it. The vesicle lies free in the uterine lumen, one wall of which is only slightly pitted. In B of figure 22 is reproduced a section of a blastodermic vesicle, measuring $90\ \mu$ by $55\ \mu$ by approximately $45\ \mu$. It is evident that this vesicle

passes through five sections of $10\ \mu$ thickness, though one of the end sections, the fifth section of the series, seems to have fallen out during manipulations of staining and mounting, since the preceding, or fourth section does not quite complete the series. This vesicle lies free in the lumen of the uterus, and there is evident only a shallow pit in the mucosa juxtaposed. In this vesicle the cells forming the roof of the segmentation cavity are relatively numerous, and are not markedly flattened, and in one an early mitotic phase is recognized. Here again cell proliferation appears to have accompanied increase in size of segmentation cavity.

The vesicle shown in C of figure 22, measuring $130\ \mu$ by $30\ \mu$ by approximately $40\ \mu$, lies free in a long, narrow fold of the uterine mucosa, in close proximity to a shallow mucosal pit, lined by cubic epithelium; the pit conforming in shape and extent to the form of the side of the vesicle presented to it. Therefore, it would seem that the form of the vesicle as seen in sections of the fixed material is essentially the same as that obtained *in vivo*. The two vesicles, typical sections of which are shown in B and C of this figure, are almost in identically the same phase of development, although their form as seen in sections differs markedly. The plasticity of the living blastodermic vesicles is no doubt such that their form is in a great measure dependent on the shape of the mucosal fold in which they are lodged. In D of figure 22, there is reproduced a section of a blastodermic vesicle which points to a stage of development which is slightly more advanced than that shown in previous figures. The vesicle measures $100\ \mu$ by $70\ \mu$ by approximately $50\ \mu$. The roof enclosing the segmentation cavity is slightly folded; a portion of its wall is thus cut tangentially, as shown in the lower left of the figure. The segmentation cavity is distinctly larger than that shown in the preceding figures, and is bounded by a relatively large number of cells, fourteen in that portion of the roof sketched in this figure, one of which is in a mitotic phase. The mass of cells constituting the floor appears as slightly compressed, in consequence of a slight intravesicular pressure which aided in the enlarging of the segmentation cavity.

The cells forming the roof of the segmentation cavity do not appear so distinctly flattened as is the case in certain of the vesicles figured in figures 20 and 21. It would appear, therefore, that at least two factors are operative in the increase of size of the segmentation cavity after its anlage—a flattening out and consequent increase of the exposed surfaces of the enclosing cells, and secondly, a cell proliferation; and it would appear that both of these factors may be operative from the time of the beginning of segmentation cavity formation.

Early stages in the blastodermic vesicle formation in the albino rat have been previously described by Robinson, Christiani, Duval, and Widakowich; Selenka's youngest stage is slightly older than any discussed by me. My own observations are wholly in accord with those of Widakowich in so far as his account covers early stages of blastodermic vesicle formation. He discusses and figures, however, only two vesicles, obtained four days after fertilization—'Befruchtung,' in each of which the segmentation cavity presents a smooth and regular outline and is of appreciable size. The observations of the other observers who have considered these stages will be discussed in connection with a very brief presentation of much more comprehensive observations on the mouse in similar stages of development. Of these latter, those of Sobotta ('03) are based on abundant and apparently well fixed material. Sobotta begins his discussion with the consideration of three ova taken from the same mouse, the second half of the fourth day after fertilization, each of which shows beginning of segmentation cavity formation, one of which was cut in longitudinal axis and is figured in his figure 1. This ovum is interpreted as showing that the segmentation cavity arises not as a single space, but as a number of disconnected spaces, which later become confluent and form a single space. A similar observation was made by Van Beneden on the bat, a fact which Sobotta uses to support his contention that the mouse ova studied by him were of normal structure. Melissinos gives a number of figures showing early stages in the formation of the segmentation cavity in the mouse. His figures 21 and 22 (66 hours) are not unlike my own figures shown in B of

figure 20 and A of figure 22. According to this observer, the segmentation cavity arises as a single space, due to an accumulation of fluid secreted by the cells of the morula. This secretion is evidenced by globule-like droplets which are shown in his drawings as adhering to certain of the cells bounding the segmentation cavity. In my own preparations of the albino rat, I find no evidence which would lead to the supposition that the segmentation cavity does not begin as a single space nor do I find any evidence of secretory globules as described by Melissinos. Selenka has described quite fully two blastodermic vesicles of the mouse, lying free in the uterine lumen. His account of their structure, supported by two somewhat diagrammatic figures, is as follows: The wall of the vesicle is formed by a layer of covering cells—'Deckzellen'—constituting a covering layer—'Deckschicht' or 'Rauber's layer.' The space enclosed by this layer, for about one-third to one-half of its extent, contains the 'formative cells,' for the remainder it contains fluid. The covering cells and formative cells are said to be separated by a sharp line. The formative cells are in all parts separable into two fundamental germ layers. An inner layer, bordering the cavity and constituting the entoderm, is said to be composed of cells possessing more deeply staining nuclei, irregular outline, with tongue-like processes which extend into the cavity, and a granular protoplasm; further, of cells which are more clear, more peripherally placed, and which constitute the ectoderm. Each of these fundamental germ layers consists of a single layer of irregularly formed cells. According to the observations of Selenka, therefore, the floor of the vesicle consists of three layers of cells; an outer covering layer—'Deckschicht' or 'Rauber's layer'—an inner layer of entodermal cells, and an intermediate layer of ectodermal cells. Jenkinson's account reads as follows: "There are present (1) an outer layer, one cell deep, of trophoblast, which is continuous over (2) an inner mass which becomes differentiated into the embryonic epiblast and the hypoblast, and which is quite distinct from the overlying trophoblast, as my specimens invariably show." In Jenkinson's figures 1 and 2, giving early stages of the blastodermic vesicle, there is not shown a differentiation of the inner

mass into ectodermal and entodermal cells; the outer layer, covering layer, Rauber's cells, or trophoblast, is clearly differentiated from the inner mass by a distinct space. Duval has recognized in early stages of blastodermic vesicle formation of the mouse and rat, in the thicker part of the vesicle, entodermal and ectodermal cells. The former are of irregular form, possess granular protoplasm and are said to possess the property of ameboid movement. The remaining cells are recognized as ectoderm; a distinct covering layer is not recognized. In Christiani's figures (rat), which are, however, so diagrammatic as to be of little value and are evidently drawn from poorly fixed material, entodermal cells, ectodermal cells, and covering cells may be recognized as per legends. Melissinos (mouse), while not describing definitely a peripheral or covering layer, states that outer cells of the thicker pole, like the cells enclosing the segmentation cavity, stain less deeply than do the more centrally placed cells. In earlier stages of vesicle formation, neither in figures nor in text as given by this observer, do I find reference to a differentiation into ectodermal and entodermal cells. The observations of Selenka, Duval, Robinson, Jenkinson, and others, bearing on the structure of the blastodermic vesicle of the mouse and the rat in early stages of development have been so thoroughly and critically reviewed by Sobotta that an extended discussion has here been deemed unnecessary. It may here suffice to say that while Sobotta's observations were made and his discussions based on ova obtained from the mouse, my own observations made on the albino rat are in agreement with his and support the contention that in early stages of blastodermic vesicle formation a differentiation of the thicker part or the floor of the vesicle into a covering, Rauber's cell, or trophoblast layer, and a further differentiation into ectodermal and entodermal cells, is not to be made: we differ in our accounts of the beginning of the formation of the segmentation cavity. An outer or covering layer is suggested in certain of my own preparations, most clearly in that sketched in E of figure 20. However, a uniform difference in structure and reaction to staining reagents of the outer layer of cells is not present in my own preparations. None of my own preparations gives evidence of

such an early differentiation of ectoderm and entoderm as given by Selenka, Duval, Christiani, and others. Cells of irregular outline with tongue-like projections, such as figured by Selenka and Duval I have not observed. The cells constituting the floor or the thick part of the vesicle all present essentially the same structure, while the segmentation cavity, as soon as it presents appreciable size, shows a smooth and regular outline. In figures 1 and 2, of Widakowich's communication, excellent figures of early stages of blastodermic vesicles of the albino rat, there is presented no evidence of a trophoblast layer nor a differentiation of ectodermal and entodermal cells. My own figures, 20 to 22, were drawn with the aid of camera lucida at a magnification of 1000 diameters and with the use of an intense Welsbach light. They are reduced five times in reproduction. With the exceptions of cell outlines, which as sketched do not in the preparations fall in the same optical plane, and are sketched more sharply than is perhaps warranted, the figures portray quite accurately the structural appearances presented, so far as may be with the use of a single color.

BLASTODERMIC VESICLE, BLASTOCYST, OR GERMINAL VESICLE

The material on hand is listed in table 6.

TABLE 6

RECORD NUMBER	AGE	NUMBER OF VESICLES
75	5 days, 15 hours	6
91	5 days, 16 hours	2
88	5 days, 21 hours	7
89	5 days, 21 hours	6
73	6 days	10
74	6 days	5
99	6 days	6
100	6 days	10
104	6 days	6 Total 58

During the sixth day, the blastodermic vesicle of the albino rat increases in size relatively rapidly. The greater portion of its wall is, at this stage, composed of a single layer of flattened cells. The vesicles are not as yet attached to the uterine wall, though the uter-

ine mucosa shows a distinct reaction to their presence. Localized thickenings of the uterine mucosa, sufficient to cause localized swellings of the uterine tube, indicating the position of the ova, are evident. I have experienced more difficulty in successfully fixing the vesicles during this stage than any of the earlier or later stages studied. Although my material contains 58 vesicles of the stage under consideration, none of them may be regarded as being well fixed, and the majority of them are so folded as a result of contraction during fixation that they are of little value as objects for especial study. That the vesicles are still unattached to the uterine wall is readily determined by the fact that the shrivelled vesicles are found lying free in the depressions of the uterine mucosa, lined by a low cubic epithelium, intact throughout, and retaining its normal relation to the mucosa. The molding in these mucosal depressions no doubt gives the size of the respective vesicles as *in vivo*.

It is not my purpose at this time to consider more than superficially the changes affecting the uterine mucosa during ovum implantation in the albino rat. It is hoped that this may be the subject of a future communication. It is the purpose in the present communication to confine consideration to the development of the ovum itself. Many of the observations recorded by Burekhard on the implantation of the ovum of the mouse and the formation of the decidua, I find equally adapted to similar phenomena in the albino rat. Differences are to be observed in certain details which it is not the purpose to enter into here. Grosser gives a number of excellent figures (67 to 70, and 112 to 116) showing implantation and decidua formation in the albino rat; to these the interested reader is referred for the present. The thickening of the mucosa affects primarily its antimesometrial portion. During this process of thickening, the mucosal fold in which the ovum primarily finds lodgment, becomes deepened and converted into a funnel shaped crypt communicating with the uterine lumen, and surrounded by the 'Eibuckel,' or oval fold. Burekhard's schematic figures (text figures 2 to 4) may be consulted to make the phenomenon intelligible.

In figure 23, there are reproduced representative sections of five blastodermic vesicles falling to the end of the sixth day after insemination. None of these five vesicles can be regarded as well fixed. All show a certain amount of distortion, much more evident were the entire series of each of the respective vesicles shown. The form of the blastodermic vesicle of the albino rat at this stage of development, as indicated by the molding of the uterine mucosa, is ellipsoid. Their size as *in vivo*, when distended and of regular outline, again as indicated by the molding of the uterine mucosa, is slightly larger than would be



Fig. 23 Sections of blastodermic vesicles or blastocysts of the albino rat. $\times 200$. A and C, rat No. 99, 6 days; B, D, E, rat No. 100, 6 days. *y.ent.*, yolk entoderm; *p.ent.*, parietal layer of entoderm; *p.ect.*, parietal or transitory ectoderm.

supposed from the drawings presented. By reason of this distortion, exact measurements of size cannot be given.

In A of figure 23, there is reproduced that portion of one of the sections of a blastocyst (rat No. 99, 6 days) which passes through its floor; the thin roof of this vesicle was so folded that its inclusion in the drawing was deemed undesirable. However, its floor or the germinal disc, seems to have retained its normal form and structure, presenting when traced through the series a regular concavo-convex, discoidal form. It consists in the main of three layers of cells of polyhedral type; toward the border of the disc, of two layers of somewhat flattened cells, the peripheral layer being continuous with the single layer of cells

forming the roof of the vesicle, not shown in the figure, and known as the parietal or transitory ectoderm. In the floor or germ disc, there is evident a single layer of cells bordering the segmentation cavity or blastocele and possessing a more granular protoplasm, which stains a little more intensely in Congo red. Their differentiation and characteristic reaction to staining agents is at this stage of development not quite so distinct as in slightly older stages. This layer of cells, similar to that described by Sobotta for the blastodermic vesicle of the mouse in essentially the same stage of development, he has termed the yolk entoderm, 'Dotter entoderm,' a designation which is here followed. In the more superficial layer or layers of cells no characteristic differentiation is observed. In no portion of the floor of this vesicle was a distinct covering or trophoblast layer recognized.

In the vesicle, a section of which is reproduced in B of this figure (rat No. 100, 6 days), the floor or germ disc presents essentially the same structure as that shown in A. The vesicle shown under B, was also folded, especially its roof, which was drawn to one side and was thus not cut through its entire length in the section figured. Furthermore, the section chosen for drawing does not pass quite through the center of the germ disc, but a little nearer to one of its edges, which probably accounts for the fact that there is recognized for the greater part only a single layer of cells, superimposed over the yolk endoderm, which layer is continuous with the parietal or transitory ectoderm forming the roof of the vesicle. The cells forming the yolk entoderm constitute a single layer and are quite distinctly differentiated; one of the cells shows a mitotic phase. The roof of the vesicle formed by the parietal or transitory ectoderm, is composed of a single layer of flattened cells with flattened nuclei, the form and structure of which is more correctly shown in the right half of the roof wall, which in the section is cut transversely, while the left half, owing to the folding, is shown as cut obliquely.

In C of figure 23 (rat No. 99, 6 days), there is shown a greatly compressed blastodermic vesicle, taken from a series of cross sections of the uterine horn. In this figure there is reproduced the fifth of a series of 10 sections of 10 μ thickness; therefore,

the third dimension of the vesicle is approximately 100 μ . It is evident that had this vesicle been cut in a favorable plane at right angles to the present series, or parallel to the mesometrial plane, its form would have approached that of a circle. I have in my possession one vesicle of this stage of development, similarly compressed, cut parallel to the plane of compression, in which almost the entire roof falls within a single section of 10 μ thickness. The structure of the vesicle shown in C is very similar to that shown in A and B of this figure. The normal form of this vesicle is quite readily reconstructed from a study of the series of sections into which it has been cut. The cells of the yolk entoderm are evident. The parietal or transitory ectoderm constituting the roof consists of a single layer of much flattened cells, with relatively few nuclei, having, as seen in cross section, a long ovoid form, which, when seen in surface view present a regular, nearly circular outline (see lowermost nucleus in the figure). In similarly compressed vesicles cut parallel to the plane of compression, the germ disc may appear as consisting of three to four layers of cells. In an imaginary section passing in a plane at right angles to that figured in C, and having perhaps a slight obliquity, the germ disc would appear as if much thicker than that shown in A and B of this figure. Such sections may readily lead to false conclusions.

It seems evident from a study of the material at my disposal that during the sixth day after the beginning of insemination in the albino rat, the blastodermic vesicle or blastocyst, which has its anlage in the latter part of the fifth day, enlarges relatively rapidly; this largely owing to a distension of the segmentation cavity or blastocele. This enlargement is accompanied by a flattening and extension of the enclosing roof cells and by a rearrangement of the cells of the floor, which is reduced in thickness to a discoidal area, the germinal disc or germ area, forming about one-fifth to one-sixth of the wall of the vesicle and consisting of two or three layers of cells. During the rearrangement of the cells which constitute the floor of the vesicle, those adjacent to the segmentation cavity or blastocele differentiate to form the anlage of the yolk entoderm. The remaining cells of the ger-

minal disc, having all essentially the same structure, are of irregular polyhedral form and are mutually compressed. To designate them as a distinct germ layer at this stage seems inappropriate. A differentiation into a layer of covering cells and a layer of formative ectoderm (Selenka) is not to be made. Active cell proliferation as evidenced by mitotic figures does not appear to accompany this enlargement of the vesicle. This phenomenon seems rather to be accomplished by a rearrangement of the cells constituting its floor, however, primarily by an extension and consequent flattening of the cells forming the roof of the vesicle. A similar stage is shown for the mouse by Sobotta ('03) in his figures 3, 4, and perhaps 5, of mouse vesicles from the fifth day after fertilization—'Befruchtung'. Sobotta had at his disposal much more perfectly fixed vesicles than my material contains. The structure of these vesicles as given by this observer, both as depicted in figures and text, is very similar to the presentation given by me. He also recognizes in this stage the anlage of the yolk entoderm. Figure 30, accompanying the account of Melissinos (mouse, 84 hours) presents a similar stage, although he figures fairly distinctly a layer of covering cells, which if I read him correctly, however, is of only transitory existence. None of the figures given by Robinson and Jenkinson is comparable with figures A, B, C, of figure 23 of this account.

In D, of figure 23 (rat No. 100, 6 days) there is reproduced a section of a blastodermic vesicle which on superficial study presents a somewhat later stage of development than those shown in A to C, of this figure. It is, however, only very slightly older than the three vesicles discussed. Vesicle D, cut in good longitudinal direction, is in reality much more folded than appears from the section figured. Its floor or germ disc is compressed in a plane parallel to that of the plane of section, so that the germinal disc is cut obliquely and not transversely, and thus appears thicker in the section than it in reality is. A distinct layer of covering cells, continuous with the cells of the parietal ectoderm, is evident. Such a layer of covering cells is figured by Selenka, Jenkinson, and Duval. The yolk entoderm has differentiated and extends by perhaps three cells, in the

section figured, onto the layer of parietal ectoderm. Selenka and Duval, who regard the cells of the primary entoderm as having ameboid properties, are disposed to regard the entodermal cells found lining the parietal ectoderm as having wandered from their seat of origin to the side wall of the vesicle. Sobotta sees no evidence of such wandering of the primary or yolk entodermal cells, but suggests that they are drawn to their position on the wall of the vesicle during its increase in size; their wandering, therefore, is more relative than absolute. Certain cells nearer the edge of the yolk entoderm, having attachment to the parietal ectoderm, which attachment they retain as the vesicle enlarges, are thought to be drawn from their close relation to the yolk entoderm and to appear as scattered cells lining the parietal ectoderm. Now and then, such cells may divide, resulting in further distribution. Sobotta's suggestion seems to me to be more in accord with the observed facts. In vesicle D, the roof, consisting of a single layer of flattened, parietal ectodermal cells, presents several major folds as well as minor folds. The latter particularly account for the variation in thickness of the wall of the vesicle as seen in sections. At the lower left of the figure is seen a portion of the wall as seen cut on the flat, the shape of the two nuclei here shown as seen in surface view may be compared with the long ovoid form of similar nuclei when seen in cross section.

Vesicle E of figure 23 (rat No. 100, 6 days) presents a stage that is slightly older than the other four vesicles shown in this figure. The floor of this vesicle, the germinal disc, as seen in cross section, presents the form of a triangle with its base resting on the cavity, the blastocele. When compared with the slightly younger stages this portion of the vesicle presents an increase in the number of constituent cells, arranged in irregular layers to the number of five in its thickest portion. The thickening is no doubt in part due to the slight lateral compression of the vesicle, but this does not wholly account for it. The cells constituting this thickened germinal disc are for the main of irregular polyhedral form with relatively large nuclei rich in chromatin. A distinct covering layer is not evident. On its under surface there is found a single layer of cells of yolk entoderm. The

thin-walled roof of this vesicle, the parietal or transitory ectoderm, deserves no special consideration, except to state that its variation in thickness, as seen in the section figured, is due to the plane of section—cross or oblique—of different portions of the wall, owing to slight folding. This vesicle I believe to be in stage of development and structure very similar to that shown by Sobotta ('03) in his figure 6, mouse vesicle of the first half of the sixth day, and perhaps also figure 31, of the account of Melissinos, mouse vesicle, end of fourth day, also figure 7 of Jenkinson's article who, however, describes and figures a distinct covering or trophoblast layer.

The cell rearrangement and proliferation resulting in the thickening of the floor or the germinal disc as noted in E, of figure 23, marks the beginning of a much more distinct thickening of this portion of the vesicle, partly due to cell proliferation, in part also due to the rearrangement and enlargement of the constituent cells, during which thickening process this portion of the vesicle grows outward as well as into the cavity of the vesicle, initiating the phenomenon known as the 'inversion of the germ layers' or as 'entypy' of the germ layers, to be discussed as to its anlage in the following section.

LATE STAGES OF BLASTODERMIC VESICLE, BEGINNING OF ENTYPY OF GERM LAYERS

The material at hand is listed in table 7.

TABLE 7

RECORD NUMBER	AGE	NUMBER OF VESICLES
46	6 days, 14 hours	10
54	6 days, 16 hours	9
67	6 days, 16 hours	7
24	6 days, 17 hours	3
90	6 days, 17 hours	6
72	7 days	9
80	7 days	9
92	7 days	6 Total 59

The fixation of the blastocysts of the albino rat obtained during the seventh day after insemination was much more

readily accomplished than in those obtained during the preceding day. Of the 59 vesicles of this stage obtained, many show excellent fixation. The thin wall of the vesicle is no longer so prone to fold as in the preceding stage, and does not readily retract from the uterine epithelium or mucosa, no doubt owing to a distinct adhesion of vesicle wall to the maternal tissue. It is difficult, however, so to orient the vesicles as to obtain sections of a desired plane. The general position of a given vesicle is readily determined, since the enlargement of the uterus marking its location is very evident. The vesicles are located in approximately cylindrical cavities, known as decidual crypts, which are directed toward the antimesometrial border.

These decidual crypts communicate with the lumen of the uterus, which lies eccentric and nearer the mesometrial border, by means of funnel-shaped openings. The decidual crypts or cavities are still lined with uterine epithelium, though this is now much flattened in the immediate vicinity of the vesicle and may be found in part separated from the mucosa of this region. The vesicles are now so placed that in all of them, the thicker portion, the floor of the blastodermic vesicles of younger stages or region of the germinal disc, is directed toward the mesometrial border, thus toward the still patent lumen of the uterus, while the roof of the vesicles is directed toward the antimesometrial border, thus toward the bottoms of the decidual crypts. The general direction of the decidual crypts is in the main at right angle to the long axis of the uterine horn, and directed from the mesometrial to the antimesometrial border. They may deviate, however, from the general direction at various angles and in almost any direction. The decidual crypts as seen in cross section do not as a rule present a circular outline, but appear as slightly compressed from side to side, having thus an oval outline as seen in cross section, with the long axis of this oval space as seen in cross section approximately parallel to the long axis of the uterine horn. Since the direction of the decidual crypts can in uncut material be only approximated, the obtaining of sections cut in a desired plane becomes largely a matter of chance. In a large number of my preparations the contained

vesicles are cut in an oblique plane, which may deviate only a little from the longitudinal or may approach a cross axis, while only a relatively small number of vesicles were cut favorably in the longitudinal plane, and the majority of these are in series cut parallel to the plane of the mesometrium. The vesicles on which the special consideration of this stage is based are reproduced as seen in sections, in figure 24.

Vesicle A, of figure 24 (rat No. 46, 6 days, 14 hours), is drawn from two successive sections. The upper portion of the figure



Fig. 24 Sections of blastodermic vesicles or blastocysts of the albino rat showing the early stages of entopy of the germ disc. $\times 200$. A and B, rat No. 46, 6 days, 14 hours; C, rat No. 54, 6 days, 16 hours; *ect.pl.*, ectoplacental cone or Träger; *ect.n.*, ectodermal node; *p.ect.*, parietal or transitory ectoderm; *v.ent.*, visceral layer of entoderm; *p.ent.*, parietal entoderm.

was drawn under camera lucida from one section, then by superimposing certain of the cells so as to give proper orientation, the lower half of the figure was added from the succeeding section. The slightly oblique plane in which this vesicle was cut made this procedure desirable. This relatively small vesicle seems in excellent state of fixation, as is evident from the symmetrical outline shown by the successive sections of the series. When compared with vesicle E of figure 23, though the two are separated in time of development by only a few hours, it is evident

that a distinct advance in development has taken place. The so-called floor of vesicle A, the region of the germinal disc of former stages, directed toward the mesometrial border, is markedly thickened, resulting in an outgrowth toward the mesometrial border and an ingrowth into the cavity of the vesicle. The outgrowth forms the anlage of the 'Träger' (Selenka) or the 'ectoplacental cone' (Duval), and appears to have developed largely as a result of an increase in size of the more superficially placed cells, since cell proliferation is not marked in this region. It is admitted that the critical stages are here lacking in my material. These stages appear to fall to the early hours of the seventh day, the material for which is lacking.

As may be seen from the figure, the cells constituting the anlage of the ectoplacental cone are of relatively large size with large vesicular nuclei, and are continuous at the base with the parietal ectodermal cells which form the roof of the vesicle or its antimesometrial portion. In the cell mass which extends into the cavity of the blastodermic vesicle or blastocyst in which there is recognized the anlage of the 'egg-plug'—'Eizapfen,' or 'egg cylinder'—'Eicylinder' (Sobotta) there is evident a fairly clearly circumscribed compact mass of cells, which stain somewhat more deeply than the surrounding cells and which may be designated as the ectodermal node. It represents the anlage of the true ectoderm of the embryo, as may here be stated in anticipation of further description. In all of the vesicles of this stage of development, even when cut obliquely or in cross section, this small nodule of compactly arranged cells is evident. It is circumscribed both from the cells of the ectoplacental cone as also from the cells lining the blastoceles. The metamorphosis leading to the formation of the ectodermal node will receive consideration in a brief general discussion of this stage. The cells covering the egg-plug, and surrounding the ectodermal node, so far as it extends into the blastocoele, are arranged in a single layer, forming a dome-shaped membrane, which appears as forced into the cavity of the vesicle consequent on development of the ectodermal node. This layer of cells constitutes the yolk entoderm, the anlage and differentiation of which has been previously

considered. The antimesometrial portion of this vesicle, its roof, consists of a single layer of somewhat flattened cells, the parietal or transitory ectoderm. The parietal ectoderm presents on its inner surface a few—four in the section figured—entodermal cells of irregular outline. These may be designated, after Sobotta, as cells of the parietal entoderm.

Vesicle B, of figure 24, taken from the same rat as was vesicle A (rat No. 46, 6 days, 14 hours) presents a very favorably cut vesicle, which, however, is slightly compressed from side to side, so that its form appears more nearly circular in the sections cut in the plane of the figure, than were they cut at right angles to this plane. This is especially true of the ectoplacental cone, which for the greater part appears in only two sections of 10 μ thickness, while in the plane of the figure it measures nearly 90 μ . Cognizance of this is to be taken in considering the relative size of the ectoplacental cone as shown in this figure. This vesicle is only very slightly older than that shown in A of this figure. Its ectoplacental cone is made up of a core of relatively large cells, bordered by more flattened cells, which in this preparation stain somewhat more deeply than do the more centrally placed cells. These covering cells are continuous with the cells of the parietal ectoderm. The cell mass projecting into the blastocoele is more definitely circumscribed than in the slightly younger stage shown in A of this figure. The ectodermal node appears as an oval mass composed of compactly arranged cells, and is separable on all sides from the surrounding cells. The yolk entoderm, which may now be known as the visceral layer of the entoderm (Sobotta) passes as a single layer of cells of quite regularly cubic or short columnar form, nearly about the ectodermal node to reach the base of the ectoplacental cone, extending over on the parietal ectoderm at one side (see right side of figure). A few of the cells of the parietal entoderm, three in the figure, are evident. The parietal ectoderm forming the roof or antimesometrial portion of this vesicle consists of a single layer of flattened cells, which rest on, and are adherent to the decidual tissue; the uterine epithelium lining the decidual crypt in which the vesicle is lodged having in part disappeared in the immediate region of the vesicle.

Vesicle C of figure 24 (rat No. 54, 6 days, 16 hours) presents a stage which is almost identical in development with that shown in B of this figure, though in shape these two vesicles, as seen in sections, appear quite different. The vesicle shown in C is less compressed than the one shown in B, and probably presents more correctly the form of the blastodermic vesicle or blastocyst of the albino rat at this stage of development. The ectoplacental cone presents a cylindrical outline and contains two cells showing mitotic phases, both included in the section figured. Its cells, more particularly the ones bordering the periphery, present a vacuolated protoplasm, the vacuoles containing lightly colored globules which from reaction to the stain are to be regarded as blood cells or fragments of such, which blood cells are regarded as of maternal origin. In this preparation, the decidual crypt contains a small amount of extravasated maternal blood, found in part surrounding the ectoplacental cone; also in the antimesometrial portion of the crypt in relation with the roof of this vesicle. These findings will receive further consideration in the succeeding pages. The cell mass projecting into the cavity of the vesicle, consisting of the ectodermal node and the layer of visceral entoderm is slightly larger than in the preceding stage but presents no special features deserving discussion. The vesicle in the section sketched presents very few cells of the parietal entoderm. The parietal ectoderm forming the roof of this vesicle consists of a single layer of flattened cells in the protoplasm of certain of which vacuolization is evident. Certain of the cells show inclusions of lightly staining globules of a color similar to those found in the cells of the ectoplacenta, particularly evident in the lower right of the figure in which they are represented as uncolored circumscribed areas. The color reaction of these globules is like that of the maternal blood cells and fragments of blood cells found in the decidual crypt in the immediate vicinity of the vesicle, and they are regarded as blood cells or fragments of such, taken up by the cells of the parietal ectoderm at this stage in the development of the vesicle.

The blastodermic vesicles or blastocysts figured in figure 24, represent an important stage in the development of the albino

rat, as also in a number of other rodents, in that they show the anlage of the phenomenon known as the inversion of the germ layers or entypy of the germ layers. "Inversion of the germ layers—Blätterumkehrung"—in the ova of rodents was probably first recognized by Reichert in the guinea-pig, mouse, and rat, though it was much more fully and correctly described by Bischoff as observed in the guinea-pig and a little later by Hensen, also in the guinea-pig. Further observations on this phenomenon were recorded by Kupffer in his study of the development of the field mouse, *Arvicola arvalis*, and by Fraser on the gray and white rat and the mouse. Selenka gave this question special study, and in a number of monographic communications deals with the phenomenon of Blätterumkehrung as observed in three varieties of the mouse, the white rat, and the guinea-pig. Selenka's observations have formed the basis for future work on this problem. They have been widely accepted and extensively quoted. It was he who introduced the term 'Träger' to denote the cell mass which results from proliferation of the covering cells. His own words concerning this point read as follows:

Während bei dem Kaninchenei, nach erfolgter Sonderung der formativen Furchungszellen in äusseres Ektoderm und inneres Entoderm, die gesammte Lage der äusseren Deckzellen zu einer dünnen resistenten Membran zusammenschrumpft, verdickt sich bei den Nagern mit invertirten Keimblättern der mit den formativen Zellen in Contact befindliche Abschnitt der Deckschicht unter lebhafter Zellvermehrung zu einem sphärischen oder konischen Gebilde, welches ich als 'Träger' bezeichne; * * * * Die Einwucherung dieses Trägers ins Innere der Keimblase hat zur Folge, dass die scheibenförmigen Grundblätter (Ektoderm und Entoderm) sich nicht wie beim Kaninchen zu zwei concentrischer Hohlkugeln erweitern, sondern, ehe sie noch zu dieser Gestalt gelangten, ins Centrum der Keimblase vorgeschoben, vorgestülpt und damit invertirt werden.

In a later publication, this observer also suggested the name 'Entypie des Keimfeldes' as a more comprehensive term than 'Blätterumkehrung' under which may be included types with inversion of the germ field without actual inversion of the germ layers. In later years Duval, Christiani, Robinson, Jenkinson, Sobotta, Kolster, D'Erchia, Spee, Burckhard, Melissinos, Widakowich, Lee and others have studied the earlier developmental

stages of rodents presenting the so-called inversions of the germ layers. O. Hertwig in his chapter "Die Lehre der Keimblätter" gives a brief résumé of our knowledge of the inversion of the germ layers as observed in certain rodents, noting that three main modifications are to be observed. The first and simplest, as found in the field mouse; the second or intermediate as found in the rat and mouse; the third and most complex as observed in the guinea-pig. Hertwig's account is based largely on the observations of Selenka, the accuracy of which is now questioned from many sides.

My own conclusions concerning the early stages of the entypy of the germ layers in the albino rat are made on stages which do not portray the very beginning of this process. The vesicles shown in figure 24, in which this process is well initiated, however, present appearances, on the basis of which certain conclusions may be drawn. It is the contention of Selenka that the Träger or ectoplacental cone is developed as a result of proliferation of covering or Rauber's cells, superimposed on the formative cells of the germ disc. He is followed in this view by Jenkinson, who states that "At a certain stage this proximal trophoblast (the so-called Rauber's cells of the rabbit) certainly becomes very thin, but it never wholly disappears, and soon thickens again to form the Träger, or, to use a modern expression, trophoblastic syncytium, which is destined to play an all-important part in the formation of the placenta." The account of Melissinos is difficult to follow, owing to his application of the term 'Rauber-sche Schicht.' The outer layer of the blastocyst in the region of the germinal disc is said to have a transitory existence and to disappear almost completely in the earlier stages of blastocyst formation. In a later paragraph he states, "dass nur die Rauber-sche Schicht existiert und sogar in den folgenden Stadien mit zahlreicheren Kernteilungsfiguren, und dass sie den Placentar-conus liefert." Attention has previously and on a number of occasions been called to the fact that in the albino rat I have not been able to differentiate a distinct covering layer—Deckschicht or Rauber's Schicht (Selenka); trophoblast layer (Jenkinson)—and have expressed myself as wholly in accord with Sobotta's

observations on the mouse egg as concerns this point. He has critically reviewed Selenka's and Jenkinson's contentions as to the participation of the covering layer in the formation of the Träger or ectoplacental cone, reaching the conclusion that there is no evidence in support of this. In accord with Duval—and in this I concur—he states: “Die mesometrale Spitze des ‘Trägers Selenkás’ ist, wie auch Duval richtig bemerkt, sogar ganz auffällig arm an Mitosen.” The anlage of the ectoplacental cone or Träger, it would appear to me, is primarily the result of enlargement of its constituent cells, this enlargement of cells involving the more peripherally placed cells of the somewhat thickened germinal disc. In none of my preparations showing early stages in the formation of this structure are mitotic figures evident. Grosser in his figures 67 and 113, shows a germinal vesicle of the albino rat of $6\frac{1}{2}$ days in its normal position in the decidual crypt. The vesicle there figured is about identical in time and stage of development to those figured by me in figure 24. In his figures, the Träger (*Tr.*) is represented as consisting of relatively few cells in which no mitoses are evident. In slightly older stages after the means of nutrition of the vesicles is improved through ingestion of maternal blood cells (Sobotta) mitotic figures may be observed in the ectoplacental cone, as shown in C of figure 24. In the rat as in *Mus sylvaticus* and the guinea-pig (Selenka) the ectoplacental cone arises as a solid mass of cells; in *Arvicola arvalis* (Kupffer) it is at first a hollow structure and is in part formed by invagination; in the white mouse (Sobotta) the form of this cell mass may vary greatly and may be solid or penetrated by a mere slit or again by a more extensive cavity.

The earlier stages in the formation of the egg-plug or egg-cylinder I have not been able to follow. In the youngest stage showing this, at my disposal, A of figure 24, it consists of a central node of compactly grouped cells, of polyhedral form, quite definitely demarked from the surrounding cells, and very generally of oval form. This mass of cells I have designated the ectodermal node. In Grosser's figures (67 and 113, e, *Ec*) an identical structure may be observed, designated as ‘Ectoderm der Em-

bryonanlage.' The same may perhaps be observed in figure 26, plate 14, of Selenka's account. In figures 26, 28, 31, and 33 of Christiani's contribution this may be postulated, though his figures are useless for a close comparison. Duval does not figure this stage. Sobotta's ('03) figure 7, and figure 33 of the contribution of Melissinos, appear to give a corresponding stage for the mouse, but in neither of these figures is the 'ectodermal node' so clearly depicted as in Grosser's and my own figures, at least not until a somewhat older stage. Figure 6 of Sobotta ('03) may very probably be regarded as representing an intermediate stage between that shown in E of figure 23 and in A of figure 24. By a proliferation of the cells of the germinal area as shown in the former figure a stage resembling that shown in Sobotta's figure 6, is readily postulated. That the formation of the ectodermal cells is in part due to rearrangement of the cells of the germinal area I believe to be the case, since cell proliferation is not marked in this stage. The enlargement of the more peripheral cells of the germinal area, leading to the anlage of the ectoplacental cone, would of necessity cause the forming ectodermal node to force the yolk entoderm into the cavity of the vesicle, and thus form the anlage of the egg-plug and initiate the phenomenon of entypy of the germ layers. O. Hertwig, in describing the inversion as observed in the mouse and rat, after considering the formation of the Träger through proliferation of the cells of the Deckschicht, following here Selenka's account, states, referring to the Träger, "Durch ihn wird der formative Teil des Ektoblasts nach dem Centrum der Blase vorgetrieben, wobei er sich in eine allseits abgegrenzte Epithelkugel umwandelt." And again, in referring to the development of the guinea-pig, he states: "Wie bei Maus und Ratte zieht sich das formative Ektoderm zu einer Epithelkugel zusammen." Hertwig thus appears to regard the formation of the 'Epithelkugel,' the ectodermal node, as in part at least developed owing to a rearrangement of the cells of the germinal disc. After the formation of the egg-plug or egg-cylinder that portion of the yolk entoderm which covers it is designated by Sobotta as the visceral layer of the entoderm. The scattered entodermal cells, attached

here and there to the inner surface of the parietal ectoderm, in the albino rat at no time forming a continuous layer, he has designated as the parietal entoderm. He is followed in this by Widakowich. This nomenclature has been used by me in the sense employed by Sobotta. The parietal or transitory ectoderm (Kolster's 'feinfasserrige Haut') forming the roof or antimesometrial portion of the vesicles, is constituted of a single layer of flattened cells, which in the rat show no regional differentiation. The resorption of maternal blood, incidentally noted with reference to cells of the ectoplacental cone and certain of the cells of the parietal ectoderm in connection with vesicle C of figure 24, to which phenomenon attention has been drawn by Sobotta and Kolster for the mouse, will receive further consideration in the discussion of older stages.

DEVELOPMENT AND DIFFERENTIATION OF THE EGG-CYLINDER

The material at hand is listed in table 8.

TABLE 8

RECORD NUMBER	AGE	NUMBER OF OVA
17	8 days, 17 hours (?)	2 (not all cut)
35	8 days, 18 hours (?)	6
21	7 days, 16 hours	10
66	7 days, 16 hours	7
27	7 days, 17 hours	7
89	7 days, 20 hours	5
81	7 days, 22 hours	7
94	8 days	7
95	8 days	9
96	8 days	5

For the stages showing the development and differentiation of the egg-cylinder in the albino rat I am able to present a series of stages which follow one another in close succession. The figures presented are in themselves so elucidative that an extended description is obviated. The stages under consideration fall within the eighth day after the beginning of insemination, judging from the great majority of the specimens at my disposal, although two rats (Nos. 17 and 35) killed in the latter

half of the ninth day, contained stages which are younger than nearly all of those obtained the latter half of the eighth day. I am unable to state whether this is owing to a retardation in the rate of development of the ova in rats Nos. 17 and 35, or due to an error of record. The record gives date and hour of insemination and of killing, and I have no reason to doubt its accuracy. However, the two rats in question give the only instances of marked deviation from what appears as a normal rate of development as presented by the bulk of my material. Sobotta ('11) has called attention to the difficulty of obtaining successively staged material in the mouse, and cites Kolster as contending: "Man könne auf die Altersbestimmung gar nichts geben." During this stage of development the decidual crypts lodging the ova are deeper than in the preceding stage, their mesometrial portion being narrower, though they are not as yet separated from the uterine lumen. The orientation of the decidual crypts and the contained egg-cylinders is perhaps more readily made than in slightly younger stages, though not definitely enough to insure the cutting of sections in a given plane. Sections of the egg-cylinder cut in the longitudinal plane may be obtained by cutting parallel to the plane of the mesometrium or at right angles to the same. However, it is still largely a matter of chance as to whether the sections obtained pass through the midplane or at an angle thereto.

In figure 25, there are reproduced representative sections of three germinal vesicles taken from the same uterus (rat No. 35, 8 days, 18 hours) which show three closely approximated early stages in the development of the egg-cylinder. None of these three vesicles is cut in exactly the mid-longitudinal plane; especially is this true of the ends of the vesicles. Furthermore, the antimesometrial portion of each, lower part of the figure, composed of the thin-walled parietal ectoderm, shows a certain amount of folding, so that a portion of each wall is cut *en face* instead of *en profile*. The appearances here presented by the antimesometrial portion of these vesicles is not to be confused with a 'giant cell' formation of this portion of the roof of the vesicle, described by Sobotta in his earlier publications, but corrected and retracted

in his later communications. Vesicle A, figure 25, when compared with vesicle C of figure 24, shows only a slight difference in degree of development. Vesicle A is of more elongated and of more distinctly cylindrical form. Its thin-walled portion (an-

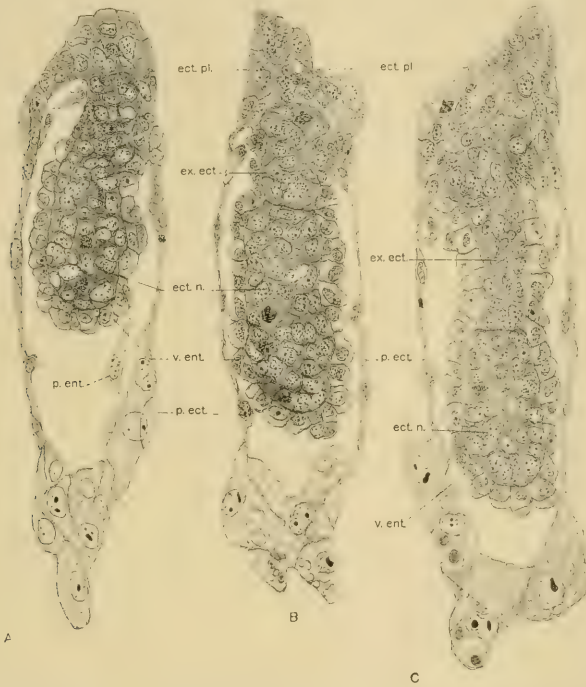


Fig. 25 Longitudinal sections of blastodermic vesicles of the albino rat, showing entypy or inversion of germ layers with early stages in egg-cylinder formation. The ectoplacental cone of each is not cut through its entire length and the lower portion of each vesicle is slightly folded. $\times 200$. A, B, and C, rat No. 35, 8 days, 18 hours, after insemination. To fit properly into the entire series these three vesicles should be from the early hours of the seventh day after insemination. *ect.pl.*, ectoplacental cone or Träger; *ect.n.*, ectodermal node; *ex.ect.*, extraembryonic ectoderm, early stage of its ingrowth shown in vesicle A; *p.ect.*, parietal or transitory ectoderm; *v.ent.*, visceral layer of entoderm; *p.ent.*, cells of parietal entoderm.

timesometrial portion) is longer, its cavity more extensive; this is owing to a further flattening of the cells of the parietal or transitory ectoderm. In vesicle A in the section preceding the one figured, the ectoplacental cone is thicker by about two

rows of cells than in the one figured; the section figured not passing through the center of this structure. In vesicle A, the ectodermal node, which is distinctly demarked, no longer rests against the base of the ectoplacental cone, as in C of figure 24, but has been forced farther into the cavity of the vesicle by reason of proliferation of the cells at the base of the ectoplacental cone, resulting in the formation of a nearly cylindrically formed column of compactly arranged, polyhedral-shaped cells interposed between the ectodermal node and the base of the ectoplacental cone, but merging into the latter without sharp demarcation. To this mass of cells the name of extraembryonic ectoderm has been given by Widakowich. However, under this term this author includes also the cells of the ectoplacental cone. The ectodermal node is of larger size than in the slightly younger stage, C of figure 24, the result of cell proliferation. In the section sketched, three mitotic figures are evident in this structure. Its cells are of polyhedral shape, and show no definite arrangement. The ectodermal node and the extraembryonic ectoderm, to the base of the ectoplacental cone, together form a cylindric structure enclosed within a layer of visceral entoderm, which in the section figured is in part cut tangentially, and thus simulates an epithelium consisting of two layers of cells, but consisting in reality of a single layer of cells. Ectodermal node, extraembryonic ectoderm, and the layer of visceral entoderm together form a structure of cylindric shape which extends into the cavity of the vesicle for a distance about one-half its extent, forming the anlage of the egg-cylinder (Sobotta). Very few parietal entodermal cells are to be found on the inner surface of the parietal ectoderm. Vesicles B and C of figure 25 differ from that discussed under A, only to the extent to which the ectodermal node has been forced into the cavity of the vesicle owing to further growth of the extraembryonic ectoderm, to the extent that in C, the elongated egg-cylinder approaches the antimesometrial end of the cavity of the respective vesicle. Ectodermal node and extraembryonic ectoderm are at this stage distinctly demarked, though in close apposition. An indenture from the surface at the region of the union of these structures

with a consequent infolding of the layer of visceral entoderm is not as a rule evident, if so, only very slightly, as to the left in B; such infolding of the visceral entoderm is not regarded as having special significance. These structures, ectodermal node and extra-embryonic ectoderm, are appropriately referred to as ectodermal cylinder by Widakowich, and with the visceral entoderm, as constituting the egg-cylinder of Sobotta.

Under A of figure 26 (rat No. 17, 8 days, 17 hours), there is shown a representative section of a vesicle which is only very slightly older than that shown under C, figure 25. This vesicle was exposed, by teasing away, after fixation, the decidual tissue forming one side of the decidual crypt; this being done before embedding, so as to admit of orientation of its long axis. This accounts for the collapsed state of the thin wall of the vesicle and its slight folding, also for the fact that the ectoplacental cone is reflected upon itself. The egg-cylinder is cut in a very favorable longitudinal plane. In its antimesometrial portion, lower part of the figure, the cells of the ectodermal node now show definite arrangement in practically a single layer, with alternating nuclei. The beginning of a central cavity is evident with reference to which the cells are arranged. This cavity is the anlage of the 'Markammionhöhle' of Selenka, more appropriately known as the antimesometrial portion of the proamniotic cavity. The cells forming the wall of the ectodermal vesicle (Ektodermblase, Selenka), derived from the ectodermal node, may now be known as the primary embryonic ectoderm (Widakowich). The extraembryonic ectoderm in the mesometrial portion of the egg cylinder has differentiated to form a relatively long irregularly cylindric structure, continuous with the base of the ectoplacental cone, composed of irregular polyhedral cells, compactly arranged and showing as yet no definite orientation. In these cells active proliferation is evidenced by numerous mitoses. The egg-cylinder is covered by a single layer of cells of the visceral entoderm. Over the antimesometrial end of the egg-cylinder, the entodermal cells now present a cubic or thick pavement form, while along the sides of the egg-cylinder they are of columnar form, especially long in the region where the primary



Fig. 26 Longitudinal sections of egg-cylinders of the albino rat, showing the anlage of the antimesometrial and mesometrial portions of the proamniotic cavity. $\times 200$. A, rat No. 17, 8 days, 17 hours; B and C, rat No. 81, 7 days, 22 hours, after insemination. A, shows the very beginning of the development of the antimesometrial portion of the proamniotic cavity developing within the ectodermal node; C shows the beginning of the proamniotic cavity developing in the extraembryonic ectoderm; *ect.pl.*, ectoplacental cone or Träger; *p.ect.*, parietal or transitory ectoderm; *ex.ect.*, extraembryonic ectoderm; *v.ent.*, visceral entoderm in B and C, the cells of this layer showing the anlage of the three zones showing absorption of maternal hemoglobin; *a.met.pr.*, antimesometrial portion of proamniotic cavity, developing in the ectodermal node; *pr.emb.ect.*, primary embryonic ectoderm; *ect.ves.*, ectodermal vesicle; *met.pr.*, mesometrial portion of the proamniotic cavity, developing in the extraembryonic ectoderm.

embryonic ectoderm and the extraembryonic ectoderm meet. The special cytomorphosis undergone by the columnar cells of the sides of the egg-cylinder, in contradistinction to those of the antimesometrial end, will be considered in later pages. The visceral layer of the entoderm extends to the base of the ectoplacental cone, in part passing over onto the layer of parietal ectoderm. In the section figured, cells of the parietal layer of the entoderm are not evident. The ectoplacental cone has grown in length in the direction of the lumen of the uterus or the mesometrial border. In the great majority of my preparations this structure is slightly compressed from side to side, so as to be broader in a plane parallel to the long axis of the uterus. In vesicle A, it is cut at right angles to the long axis of the uterus, thus appears as much narrower than in the other two vesicles of figure 26, which were cut in a plane parallel to the plane of the mesometrium. The increase in size of the ectoplacental cone is the result of active cell proliferation. Mitotic figures to the number of one, two or three, may now be observed in nearly every section of this structure. The parietal or transitory ectoderm, continuous with the base of the ectoplacental cone, has been reduced by this stage to a thin, practically homogeneous membrane, presenting scattered, flattened nucleated cells on its inner surface. This thin membrane is now quite firmly adherent to the wall of the decidual crypt, throughout nearly its whole extent.

Under B of figure 26 (rat No. 81, 7 days, 22 hours) there is shown a representative section of a vesicle which is slightly more advanced in development than that shown in A of this figure. The antimesometrial portion of the proamniotic cavity, the anlage of which was shown in the preceding stage, is well established. Its wall, consisting of primary embryonic ectoderm is composed of a single layer of cells with nuclei in essentially the same plane. The primary embryonic ectoderm forms a closed vesicle (Ectodermblase, Selenka) distinctly demarked from the extraembryonic ectoderm. In this as in the preceding stage the extraembryonic ectoderm forms a long cylindrical structure continuous at its mesometrial end with the base of the

ectoplacental cone. The cells are of irregular polyhedral form, compactly grouped, showing as yet no definite arrangement. Cell proliferation as evidenced by mitoses is active, amply accounting for the increase in length of this structure. The visceral entoderm encloses the long egg-cylinder as a single layer of cells and is continuous at its base with the parietal entoderm, well shown at the left of the figure. The ectoplacental cone of this vesicle is very favorably cut in a plane parallel to the long axis of the uterus. This vesicle was unusually well fixed and may be regarded as showing normal relations of the thin membranous wall, derived from the parietal ectoderm, and of the egg-cylinder, which reaches quite to the antimesometrial end of the vesicle.

Vesicle C of figure 26, obtained from the same uterus as was vesicle B (rat No. 81, 7 days, 22 hours), differs from that shown under B, in that it presents the anlage of a mesometrial portion of the proamniotic cavity. In the extraembryonic ectoderm, near its junction with the base of the ectoplacental cone, two irregular spaces may be observed. These are distinctly evident, passing through the entire section, only in the section figured. The antimesometrial portion of the egg-cylinder is not cut quite through its center, so that the primary embryonic ectoderm of the ectodermal vesicle appears as a stratified epithelium, and the antimesometrial portion of the proamniotic cavity appears as relatively small, this owing to a slight curvature shown by this egg-cylinder. The other features presented by this vesicle are sufficiently well portrayed in the figure to obviate the necessity of further description.

In figure 27, there are shown three further stages of egg-cylinder differentiation, showing progressively older stages than shown in the preceding figure. Under A of this figure, there is reproduced a representative section of a vesicle taken from the same uterus as were vesicles B and C of figure 26 (rat No. 81, 7 days, 22 hours). The figure is not of a single section, but is combined from two sections, superimposed so as to give correct dimensions and relations. The egg-cylinder of A of this figure differs from that shown in C of figure 26, in that the mesometrial



Fig. 27 Longitudinal sections of egg-cylinders of the albino rat showing fusion of the antimesometrial and the mesometrial portions of the proamniotic cavities. $\times 200$. A, rat No. 81, 7 days, 22 hours; B, rat No. 96, 8 days; C, rat No. 94, 8 days, after insemination; *ect.pl.*, ectoplacental cone or Träger; *p.ect.*, parietal or transitory ectoderm; *ex.ect.*, extraembryonic ectoderm; *ect.ves.*, ectodermal vesicle, with wall composed of primary embryonic ectoderm, at + junction with the extraembryonic ectoderm; *a.met.pr.*, antimesometrial portion of proamniotic cavity; *met.pr.*, mesometrial portion of proamniotic cavity; *pr.c.*, proamniotic cavity; *v.ent.*, visceral entoderm; *pr.emb.ent.*, primary embryonic entoderm.

portion of the proamniotic cavity, developing in the extra-embryonic ectoderm, is of greater dimension. Two relatively large spaces, bordered by a single layer of cells of the extra-embryonic ectoderm, are to be observed. At the junction of the extraembryonic ectoderm and the ectodermal vesicle of primary embryonic ectoderm a further space of triangular outline may be seen. The primary embryonic ectoderm is arranged in the form of an oval-shaped vesicle, forming the antimesometrial end of the egg-cylinder. Its wall is relatively thin at the region of its apposition to the extraembryonic ectoderm, just below the triangular space above mentioned. This ectodermal vesicle is peculiar in that its cavity contains the remains of four cells. A study of the series of sections shows that these cells do not represent the crest of a fold of the wall of this vesicle, since they are not nearly so distinct in preceding and succeeding sections. It may only be conjectured that during the rearrangement of the cells of the ectodermal node, resulting in the formation of the ectodermal vesicle, certain of the cells became separated from the wall and remained free in the cavity. The primary embryonic ectoderm, forming the wall of the ectodermal vesicle is readily differentiated from the extraembryonic ectoderm, both by the fairly sharp definition of the ectodermal vesicle and by reason of the fact that its cells stain somewhat more deeply than do the cells of the extraembryonic ectoderm, as also the cells of the visceral entoderm. In the egg-cylinder shown under B of figure 27 (rat No. 96, 8 days) the antimesometrial portion of the proamniotic cavity, developing in the ectodermal node, and the mesometrial portion of the proamniotic cavity, developing as several discrete spaces in the extraembryonic ectoderm, have in part joined to form a single proamniotic cavity. The mesometrial portion of this cavity is still bridged by a septum of extraembryonic ectodermal cells, closing off a relatively large space found in its mesometrial portion. With the junction of the antimesometrial and the mesometrial portions of the proamniotic cavity, the primary embryonic ectoderm and the extraembryonic ectoderm become a continuous layer, the line of union of the two portions, however, remains evident and is readily recognized in all the egg-cylinders

of this and older stages, a question which will receive further consideration in following pages.

In C of figure 27 (rat No. 94, -8 days) the proamniotic cavity forms a continuous, single space. The figure presented is drawn from two sections; its greater portion, to the base of the ectoplacental cone from one section, the ectoplacental cone from another section. The junction of the membranous wall of the vesicle to the base of the ectoplacental cone, in the two sections used for the figure, was superimposed under camera lucida in joining the portions drawn from the two sections. It is believed that the drawing as presented gives correctly dimensions and relations of the different parts of this vesicle. The wall of the antimesometrial portion of the single proamniotic cavity is formed by the primary embryonic ectoderm, the cells of which are for the main of irregular columnar shape, with alternately placed nuclei. These cells are in active proliferation, as is evidenced by numerous mitoses. The wall of the mesometrial end of the proamniotic cavity is formed of a single layer of cells of the extraembryonic ectoderm; these cells are of quite regular shape with nuclei placed in about the same plane. They stain less deeply than do the cells of the primary embryonic ectoderm. In this egg-cylinder (C, fig. 27) the proamniotic cavity does not extend so near the base of the ectoplacental cone as in a number of other preparations in my possession, showing about the same stage of development; in certain of these, the proamniotic cavity extends to near the mesometrial end of the egg-cylinder.

A more definite characterization of the different parts of the egg vesicle of the albino rat at the stage of development shown in C, figure 27, end of the 8th day, seems desirable, and in doing so I shall use the terminology used by Sobotta and Widakowich. The vesicle under consideration has reached a length of 0.65 mm., and a width of 0.12 mm. Somewhat more than one-fourth of its length consists of ectoplacental cone or Träger. The cavity enclosed is derived from the cavity of the blastodermic vesicle with germ disc, the blastocele, and is termed by Sobotta and Widakowich the 'Dottersackhöhle' or yolk-sac cavity. This cavity is bounded by a thin structureless mem-

brane derived from the parietal or transitory ectoderm and the scattered cells forming the parietal layer of entoderm. This membrane is continuous with the base of the ectoplacental cone and presents scattered flattened cells on its inner surface. I have designated this thin membrane with cells on the inner surface as the parietal or transitory ectoderm (Kolster's *feinfaserige Haut*). The egg-cylinder which extends to the antimesometrial end of the yolk-sac cavity, encloses the proamniotic cavity, the antimesometrial portion of which is walled by primary embryonic ectoderm, its mesometrial portion by extraembryonic ectoderm, the two forming a continuous layer, with line of union of the two types of ectoderm evident. The uncleaved extraembryonic ectoderm is continuous with the base of the ectoplacental cone. The egg-cylinder is surrounded by a single layer of cells of the visceral entoderm, differentiated so as to consist of a portion which surrounds the antimesometrial end of the egg-cylinder in relation with the primary embryonic ectoderm; the cells of this portion being of a rather thick pavement type, constituting the primary embryonic entoderm, and further a portion which covers the sides of the egg-cylinder, with cells of a columnar type, showing special cytomorphosis. The egg-vesicles and egg-cylinders of the stage of development under consideration and for somewhat older stages show no bilateral symmetry so far as can be discerned by study under the microscope. In longitudinal sections of egg-cylinders, cut respectively in two different planes, at right angles to each other, no difference in form, relation and structure of different parts can be observed. Selenka, Kupffer, Duval, and Sobotta have previously called attention to this fact and shown that longitudinal sections of egg-cylinders may be obtained no matter whether the sections are cut parallel to the plane of the mesometrium, thus parallel to the long axis of the uterus, or at right angles to this plane. The want of bilateral symmetry is also evident in cross sections of the egg-cylinder, as may be seen from the series of sections presented in figure 28 (rat No. 27, 7 days, 17 hours). The cross-cut egg-cylinder, from several sections of which these figures were drawn, represents a stage of develop-

ment very similar to that of the egg-cylinders shown in longitudinal section in figure 26.

Widakowich, after discussing very briefly the mode of development of the egg-cylinder, discusses and figures an egg-cylinder of the albino rat, obtained $6\frac{3}{4}$ days after the last coitus. His figure 3 corresponds in stage of development very closely to that shown by me in A of figure 27. In his figures, there is presented an egg-cylinder showing the anlage of the mesometrial

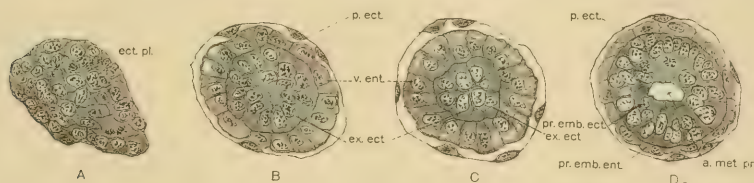


Fig. 28 A series of cross sections at different levels of an egg-cylinder of the albino rat after the anlage of the antimesometrial portion of the proamniotic cavity. $\times 200$. Rat No. 27, 7 days, 17 hours, after insemination. The sections selected for the several levels drawn, A to D, are as follows: A, middle of ectoplacental cone; B and C, through extraembryonic ectodermal portion of egg-cylinder, just below junction with ectoplacental cone (B), and just above ectodermal vesicle (C); D, through middle of ectodermal vesicle. Compare with B, figure 26, a longitudinal section of an egg-cylinder of the same stage of development; *p.ect.*, parietal or transitory ectoderm; *ex.ect.*, extraembryonic ectoderm; *pr.emb.ect.*, primary embryonic ectoderm of the ectodermal vesicle; *v.ent.*, visceral entoderm; *pr.emb.ent.*, primary embryonic entoderm; *a.met.pr.*, antimesometrial portion of proamniotic cavity.

portion of the proamniotic cavity. Emphasis is given to the fact that in the antimesometrial portion of the egg-cylinder, there may be recognized the primary embryonic ectoderm. His own words with reference to this point read as follows:

Der Schnitt zeigt nun sehr deutlich, dass sich die Zellen, die die antimesometrale Höhle so begrenzen, dass die alte Kugel- oder Eiform dieses Teiles noch zu erkennen ist—das primäre embryonale Ectoderm—intensiver färben wie die Zellen des mesometralen Abschnittes oder die des Ectoplacentarconus—das extraembryonale Ectoderm. Die Kerne zeigen keinerlei Unterschied in der Färbung, wohl aber das Plasma, dass im antimesometralen Teile von dichter Structur zu sein scheint.

This description corresponds very closely to that given by me for a similar stage. The differentiation of these two kinds of ectoderm was also recognized by Robinson, who states:

The epiblastic cylinder is closed at its distal end, the trophoblastic at its proximal, and the open ends of the two cylinders are in close apposition, but not indistinguishably fused, for the character of each portion of the ectoderm, after treatment with carmine, is still quite distinctive; the protoplasm of the trophoblast being tinged much more faintly than that of the epiblast.

Selenka, on the other hand, who has recognized in his 'Ektodermblase' with 'Markamnionhöhle' a distinctive structure, believes this to blend completely with the Träger. Since his account with reference to this point has influenced later workers, I may be permitted to quote him in the original. Referring to the 'Ektodermblase' with 'Markamnionhöhle,' he states:

Dieser Ektodermkeim, welcher von dem vorrückenden Trägerzapfen anfänglich sehr wohl abgegrenzt ist, indem beiderlei Gebilde sich in Folge der convexen Krümmung ihrer einander zugekehrten Flächen sozusagen nur in einem Punkte berühren, fließt endlich mit dem Träger vollständig zusammen, und zwar bei der Waldmaus bevor, bei der Ratte und Hausmaus aber nachdem die Markamnionhöhle entstanden war.

That the proamniotic cavity of the egg-cylinder of the albino rat has its anlage in two distinct cavities, the one developing in the ectodermal node in the antimesometrial portion of the egg-cylinder, which is the first to develop; the other in the mesometrial portion in the extraembryonic ectoderm, was recognized by Selenka (fig. 30, plate 14, E, Markamnionhöhle, E', falsche Amnionhöhle), Duval (fig. 100,) Robinson, and Widakowich (fig. 3). Corresponding stages of egg-cylinder development as presented by me in figures 26 and 27, for the albino rat, are shown by Sobotta ('02), for the mouse in his figures 12 to 14 and text figures a to f. On comparison of my figures with Sobotta's, it becomes evident that the egg-cylinder of the rat is much longer and more slender than that of the mouse. According to the account of Sobotta, the egg-cylinder of the mouse, soon after its anlage, shows by reason of a distinct transverse furrow a division into two parts, an antimesometrial portion of globular form, surrounded by a visceral layer of entoderm, corresponding to what I have designated as the ectodermal node; and a mesometrial portion which early shows the anlage of a proamniotic

cavity. A lumen is obtained in the antimesometrial portion later than in the mesometrial portion. As development proceeds, this sharp demarkation of antimesometrial and mesometrial portion is gradually lost. This, as stated in his own words, reads:

Sehen wir von dem die (der Keimhöhle zugekehrte) Oberfläche des Cylinders überziehenden Dotterentoderm zunächst ab, so sieht man, dass die Furche, welche die oben erwähnten mesometralen und antimesometralen Abschnitte in Stadium der Fig. 11 u. 12 trennte, jetzt wieder wenig deutlich ist. Es bahnt sich eine Verschmelzung beider Abschnitte wiederum an, was man am leichtesten daraus ersieht, dass bald (Fig. 14) beide Abschnitte ein gemeinsames Lumen erhalten.

With the formation of a continuous proamniotic cavity, this is bordered by a single layer of 'ectodermal cells,' with alternately placed nuclei. The cells are described as being the same throughout; neither in text nor figure does Sobotta differentiate between ectodermal cells derived from the antimesometrial portion of the egg-cylinder and those derived from the mesometrial portion. Melissinos also recognizes antimesometrial and mesometrial portions in the development of the egg-cylinder of the mouse, in his figure 34. According to this observer, the antimesometrial portion of the proamniotic cavity is the first to appear; later it appears in the mesometrial portion, the two cavities joining as development proceeds. The parts of the ectoderm derived from these two portions may be recognized, however, after a single proamniotic cavity has developed. This Melissinos states in the following words: "Trotz aller Vereinigung der beiden Höhlungen bleibt die Unterscheidung des normalen abgesonderten antimesometralen Abschnittes von dem mesometralen immer leicht zu machen, sei es durch eine klare Grenzlinie oder durch eine an der Peripherie des visceralen Dotterblattes befindliche Furche." The account of Melissinos is more in agreement with the presentations as observed in the albino rat than is that of Sobotta.

Selenka, Sobotta, and Melissinos recognize three different regions of constriction to which significance is given, in the egg-cylinder of the mouse. As stated by Sobotta, the first con-

striction is in the region of the original furrow which demarks the antimesometrial and the mesometrial portions of the egg-cylinder, the region of the primary amniotic fold; the second where the mesometrial cavity ends; and the third where the original blastodermic cavity reaches its mesometrial end. The three folds recognized by Melissinos, are characterized by the specificity of the ectoderm. Since his statement concerning this point is somewhat involved, I find it necessary to use his own words; they read as follows, referring to these folds he states:

Der eine derselben *a* liegt antimesometrial und ist der bekannte erste kugelförmige Buckel (Ektoderm) mit den länglichen, cylinder-pyramidalen oder polygonal-pyramidalen Zellen; der zweite *b* liegt in der Mitte und besteht aus kubisch-polygonalen Zellen, und der dritte Buckel *c*, aus polygonalen Zellen bestehend, liegt mesometrial und ist von dem mittleren durch Einschnürung, von der Basis des Ectoplacentarconus aber durch die bekannte Urfurche des Eieylinders getrennt, in der sich das viscerele Dotterblatt zum parietalen Dotterblatt umbiegt.

So far as I am able to determine, the account of Melissinos agrees with that given by Sobotta, as concerns the folds of the egg-cylinder of the mouse. Selenka's account need not receive special consideration.

In well-fixed egg-cylinders of the albino rat no such folds are recognized. At the line of junction of the primary embryonic ectoderm and the extraembryonic ectoderm, a slight infolding of the layers, variable in degree, is recognized. Other foldings of the wall of the egg-cylinder I have regarded as accidental and not of special significance. Therefore, I am wholly in accord with Widakowich, who has also discussed this question with reference to the albino rat and has described the low fold in the region of the junction of the primary embryonic ectoderm and extraembryonic ectoderm. Referring to that fold, he states: "Dass war die einzige konstante, bald stärker, bald schwächer ausgeprägte Einschnürung der Proamnionhöhle."

Sobotta deserves credit for having described fully the differentiation and cytomorphosis of the cells of the visceral entoderm of the egg-cylinder, and since his observations on this point apply in the main to the albino rat, they may at this time be given

consideration. During the early stages of egg-cylinder differentiation and anlage of the proamniotic cavity, the layer of visceral entoderm differentiates into a portion which is in relation with the primary embryonic ectoderm of the antimesometrial portion of the egg-cylinder, in which region the cells of the entoderm are first of short cubic shape, later of the pavement type: this portion may be regarded as forming the primary embryonic entoderm, since it forms the greater part of the entoderm of the embryo. The greater part of the visceral entoderm, that which surrounds the sides of the mesometrial portions of the egg-cylinder, consisting of extraembryonic ectoderm, differentiates into cells of the columnar type. In this latter portion, with the formation of a continuous proamniotic cavity, the entodermal cells undergo characteristic cytomorphosis. In them, as stated by Sobotta, there may be recognized three main zones: (1) a basal zone with denser protoplasm containing the nucleus; (2) a middle zone with markedly vacuolated protoplasm; (3) an outer zone in which hemoglobin granules are recognized, the latter zone staining deeply in eosin. These three zones in the cells of the visceral entoderm in the region of the extraembryonic ectoderm of the egg-cylinder may be recognized in figures 26 and 27, not so clearly as in Sobotta's colored figures, particularly his figure 17 ('03) and figure 8 ('11). However, I am able to follow closely his description in my own preparations of a somewhat older stage than thus far figured. It is Sobotta's contention that in the extravasated blood surrounding the egg vesicle, in close apposition to its thin outer wall, there may be observed many red blood cells which, though presenting normal form, show a distinctly granular content. These granules stain deeply in eosin and are in shape, size, and reaction to stain very similar to granules found in the peripheral part of the cells of the visceral entoderm. On the outer surface of the thin wall of the vesicle; on its inner surface; in the cells lining this; in the yolk sac cavity; and on the outer surface of the cells of the visceral entoderm, similar granules are found. These appearances are interpreted as showing an absorption of maternal hemoglobin by the entodermal cells of the mesometrial portion of the egg-cylinder.

Sobotta's statement concerning this point, which, owing to its importance, I quote in full, reads as follows:

Man wird diese mikroskopisch erkennbaren Verhältnisse nicht anders deuten können als in folgender Weise: Die Hämoglobinschollen, die durch die äussere Wand des Dottersackes in die Dottersackhöhle gelangt sind, werden von der Oberfläche des zylindrischen, die ganze Seitenfläche des Eizylinders überziehenden visceralen Dottersack-epithels aus resorbiert und zwar geschieht das in der Weise, dass die Hämoglobinschollen zunächst als solche in der Zelle selbst eintreten, dann aber im vacuolisierten Teil der Zelle gleichsam verdaut werden, wobei die einzelnen kleinen Schollen vorher zu grösseren Tropfen zusammenfliessen scheinen.

My own observations on the albino rat as concerns this phenomenon, more particularly as concerns the structure of the cells of the visceral entoderm in the region of the extraembryonic ectoderm, corroborate Sobotta in many particulars. This question will be again and more fully considered in a contemplated later publication dealing with the implantation and decidua formation in the albino rat. It could not be considered now without a discussion of the changes involved in the development of the decidua, a question which I am not prepared to consider fully now. It may be stated, however, that judging from my own preparations and the figures of Grosser, the extravasation of blood into the egg chamber is not nearly so extensive in the albino rat as is shown in the figures of Sobotta for the mouse.

The thin membrane which surrounds the yolk-sac cavity, which I have designated as the parietal or transitory ectoderm, is derived in development from the parietal or transitory ectoderm, and the relatively few parietal entodermal cells, as described and figured for younger stages. At the stage of egg-cylinder development under consideration—with continuous pro-amniotic cavity—this structure appears as a thin, practically homogeneous membrane with scattered, flattened nucleated cells on its inner surface. Sobotta regards these cells as derived from the parietal entoderm, the cells of the parietal ectoderm having disappeared. As concerns this, I am unable to speak with certainty, since the Congo red solution used as a double stain is not particularly favorable in differentially coloring these

cells. However, I am disposed to regard these flattened cells as derived from the parietal ectoderm. The parietal entodermal cells are never numerous in the rat, and mitotic figures are seldom observed in them. With the extension of the vesicle with the enlargement of the blastocoele, the cells of the parietal or transitory ectoderm become attenuated until they appear for the greater part as a thin cuticular membrane, and I am disposed to regard the flattened nucleated masses of protoplasm lining the inner surface of this membrane as derived from the cells of the parietal ectoderm.

Much attention has been given to certain large cells which are found in close relation with the outer surface of this thin membrane. These cells, generally referred to as giant cells (*Riesenzellen*) were, by Duval, Sobotta (earlier publications) and Grosser thought to be of embryonic origin and derived from the cells of the parietal ectoderm. Selenka, Disse, Kolster, Melissinos, Pujiula, Widakowich, and later Sobotta ('11) regard them as derived from the maternal tissue and as representing differentiated decidual cells. It is not my purpose to consider more fully these cells in the present communication, since they are by me not regarded as of embryonic origin. My own observations as concerns them agree in the main with those of Widakowich, who, in the albino rat has followed their origin from decidual cells. Since not of embryonic origin, they have been disregarded in making the figures.

I have previously, in connection with a discussion of the structure of vesicle C, figure 24, alluded to the fact that the cells of the ectoplacental cone as also the cells of the parietal or transitory ectoderm have a phagocytic action for maternal blood cells. This Sobotta has also observed for the mouse, in which he is confirmed by Kolster who has further shown that the cells of the ectoplacental cone also take up fat particles. With the ingestion of maternal blood cells by the cells of the ectoplacental cone, more particularly, with the absorption of hemoglobin by the entodermal cells of the mesometrial portion of the egg-cylinder, a period of rapid growth of the egg vesicle is initiated. To this Sobotta has called attention for the mouse; the same

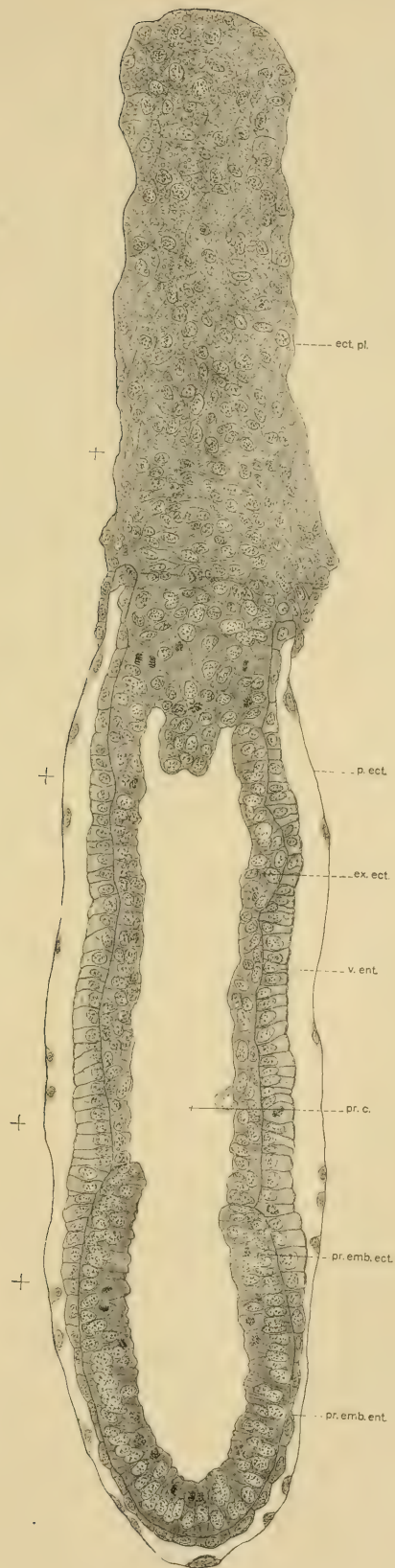
is evident in the albino rat. Indeed, Sobotta presents the far-reaching conclusion that the explanation of the phenomenon of germ layer inversion or entypy of the germ layers is to be found in the dearth of food supply of the ovum in the stages preceding the formation of more definite relations between the ova or germ vesicles with the decidua. It is thought by this observer that the inversion of the germ disc has for its purpose the increase of the absorptive surface of the visceral or yolk sac entodermal epithelium, which as a differentiated layer comes to surround nearly the whole of the egg-cylinder on completion of the inversion, and is thus increased in extent and brought in relatively close relation with the maternal blood lacunae surrounding the egg vesicle.

LATE STAGES IN EGG-CYLINDER DIFFERENTIATION AND THE ANLAGE OF THE MESODERM

In the rat series there are found 24 egg cylinders showing the stages of development considered in this section; certain of them are cut longitudinally and others cross-wise.

For the special consideration of egg-cylinder formation just prior to the anlage of the mesoderm, I present two egg-cylinders obtained during the latter half of the ninth day after insemination; one of these was cut longitudinally, the other in favorable cross-section. The egg-cylinder shown in figure 29, rat No. 40, 8 days, 17 hours after insemination, seems unusually well fixed, as evidenced by its symmetrical outline, and is cut in a very favorable plane. The sections are from a series cut at right angles to the long axis of the uterine horn. The decidual crypts lodging the egg-cylinders of this stage are by this time nearly completely separated from the lumen of the uterus, and are surrounded by a well-developed decidua. Extravasated maternal blood nearly surrounds such egg-cylinders.

Fig. 29 Longitudinal, sagittal section of egg-cylinder of the albino rat showing the final mesoderm-free stage. $\times 200$. Rat No. 40, 8 days, 17 hours, after insemination; *ect.pl.*, ectoplacental cone or Träger; *p.ect.*, parietal or transitory ectoderm; *pr.emb.ect.*, primary embryonic ectoderm; *ex.ect.*, extraembryonic ectoderm; *pr.c.*, proamniotic cavity; *v.ent.*, visceral entoderm, absorptive for maternal hemoglobin, cells showing the three zones described by Sobotta; *pr.emb.ent.*, primary embryonic entoderm.



The egg-cylinder shown in figure 29 presents a total length of 1.15 mm., a width of approximately 0.18 mm. The ectoplacental cone presents a length of 0.4 mm. and of the proamniotic cavity, 0.5 mm., of which 0.2 mm. falls to the antimesometrial portion lined by primary embryonic ectoderm. This egg-cylinder differs only in shape and size from that shown in C of figure 27, obtained 8 days after insemination. The primary embryonic and extraembryonic ectoderm lining or enclosing the proamniotic cavity are readily differentiated. The primary embryonic ectoderm, derived from the ectodermal node, constitutes a pseudostratified epithelium, composed of relatively long columnar cells, with nuclei radially placed with reference to the lumen of the proamniotic cavity, and shows active cell division, no less than 12 mitotic figures occurring in the section figured. The protoplasm of its cells stains distinctly deeper than does that of the cells of the extraembryonic ectoderm. The cells of the latter are of cubic, short columnar, or polyhedral shape, arranged in a single or double layer, with no definite arrangement of the long axes of its nuclei. It is, therefore, possible readily to distinguish—by reason of shape and size of cells, relative position of nuclei, reaction to stain of protoplasm—between the cells of the primary embryonic and extraembryonic ectoderm, and to determine the sharp line of junction at which the two types of cells form a continuous layer, a fact which will receive further consideration in dealing with the anlage of the mesoderm as observed in slightly more advanced stages. At the mesometrial end of the proamniotic cavity, the cells of the extraembryonic ectoderm become continuous with the cells at the base of the ectoplacental cone; in the region of this junction, active mitosis are often to be observed. In this egg-cylinder the visceral entoderm may readily be differentiated into two portions. The portion which surrounds the primary embryonic ectoderm to nearly the region of its junction with the extraembryonic ectoderm, consists of a single layer of broad, flattened cells which assume a cubic or short columnar shape as the mesometrial border of the primary embryonic ectoderm is approached. This portion of the visceral entoderm we have designated as

the primary embryonic entoderm. The portion of the visceral entoderm surrounding the sides of the egg-cylinder in the region of the extraembryonic ectoderm, to near the base of the ectoplacental cone, consists of a single layer of columnar cells, regularly arranged and presenting the three zones described by Sobotta. In this stage of egg-cylinder development of the albino rat, the absorption of hemoglobin granules derived from maternal blood cells, first shown for the mouse by Sobotta and Kolster, may be readily made out. In preparations stained in hematoxylin and Congo red, in and on the outer zone of the visceral entodermal cells there may be observed granules staining deeply in the Congo red, presenting the color reaction of hemoglobin. In the middle zone of these cells the protoplasm is distinctly vacuolated, while the inner zone, containing the nuclei, presents a denser protoplasm. The transitory or parietal ectoderm consists of a homogeneous membrane, closely adherent to the maternal decidua, especially along the sides of the egg-cylinder. This layer presents scattered nucleated protoplasmic masses of spindle or dome shape on its inner surface, the relations and distribution of which may be clearly seen in the figure. Attention needs yet be drawn to the ectoplacental cone of the egg-cylinder. Its relation to the maternal decidua is very intimate, so that in places, owing to blood extravasations, it is difficult to differentiate between embryonic and maternal tissue. Many of the cells of the ectoplacental cone present a vacuolated protoplasm, the vacuoles enclosing maternal blood cells. Therefore, they are distinctly phagocytic. Sobotta has also observed and described this for the mouse. Referring to a slightly older stage after the anlage of the mesoderm, his own words read as follows:

Weiterhin sehen wir im Stadium der Fig. 5 auch eine starke Verlängerung und Vergrößerung des Ectoplacentalconus, an dem im mesometralen Teile jetzt Vacuolen auftreten, die in späteren Stadien regelmässig gefunden werden und zwar erfüllt mit mütterlichen Blutextravasaten. Die Ernährung des Embryo mit mütterlichem Hämoglobin * * * * ist jetzt im vollen Gang.

Absorption of maternal hemoglobin by the cells of the ectoplacental cone appears to be established at a relatively earlier period in the rat than in the mouse.

The egg-cylinder presented in figure 29 constitutes the final mesoderm-free stage, the final stage in which no distinct bilaterality may be determined. I assume that the egg-cylinder presented in the figure is cut in the sagittal plane. This assumption is based on the fact that the primary embryonic ectoderm extends slightly farther toward the mesometrial pole on the one side than on the other. In good frontal sections one side of the egg-cylinder in this stage of development should present a mirror picture of the other side. The side on which the primary embryonic ectoderm extends farther toward the mesometrial pole, the left in the figure, is regarded as containing the caudal end of the future embryo. In the primary embryonic ectoderm of this region, it is believed, will develop the primitive streak and groove, and thus the anlage of the mesoderm. Not in all the egg-cylinders of this stage of development found in my series can the caudal end of the future embryonic area be postulated prior to the anlage of the mesoderm, and in cross-sections no such differentiation can be made. The proamniotic cavity of the egg-cylinder shown in figure 29 presents a regular and nearly smooth contour, not divisible into regions such as described for a similar stage for the mouse by Selenka, Melissinos, and Sobotta. A very slight constriction is to be observed only in the region where the primary embryonic and extraembryonic ectoderm are joined in a continuous layer. I am thus wholly in accord with Widakowich, who in describing a similar stage in one of his preparations, states: "Das war die einzige konstante, bald stärker, bald schwächer ausgeprägte Einschnürung der Proamnionhöhle," as previously quoted.

A series of figures of critical regions taken from a series of cross-sections of an egg-cylinder of a stage nearly identical with that shown in figure 29, though of a slightly smaller egg-cylinder, is given in figure 30, rat No. 42, 8 days, 16-hours, after insemination. The sections chosen for the several drawings, A to D, are from the following regions, as may be ascertained by comparison with figure 29; A, through about the middle of the ectoplacental cone; B, through the proamniotic cavity just below its mesometrial end; C, through the proamniotic cavity just above the

region of the junction of the primary embryonic and extraembryonic ectoderm; D, a little above the middle of the antimesometrial portion of the proamniotic cavity. The levels of the

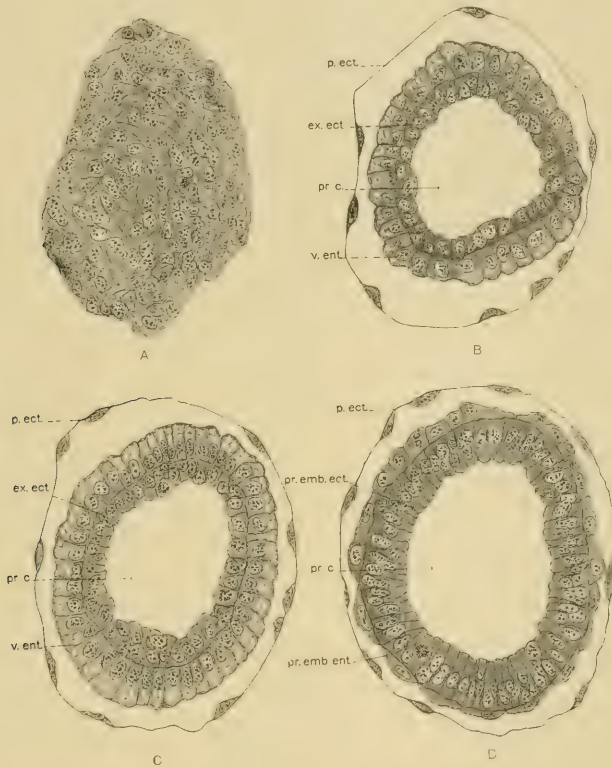


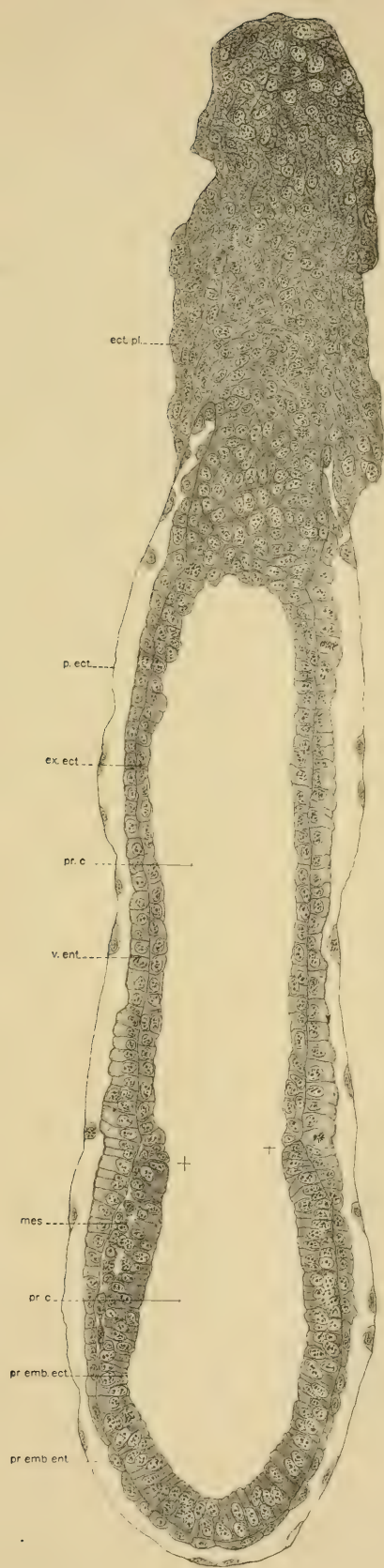
Fig. 30. Four figures from a series of cross sections of an egg-cylinder of the albino rat in the stage of development shown in figure 29. $\times 200$. Rat No. 42, 8 days, 16 hours after insemination.

The levels at which the several sections drawn were taken is approximately indicated by the several crosses found to the left of figure 29. A, middle of ectoplacental cone; B, ectoplacental end of the proamniotic cavity; C, just above level of junction of the primary embryonic and extraembryonic ectoderm; a little above the middle of primary embryonic ectoderm. The want of any definite bilateral symmetry of albino rat egg-cylinders of this stage of development is shown by this series of sections; *p.ect.*, parietal or transitory ectoderm; *ex.ect.*, extraembryonic ectoderm, surrounding mesometrial portion of proamniotic cavity; *pr.emb.ect.*, primary embryonic ectoderm; *v.ent.*, visceral entoderm; *p.emb.ent.*, primary embryonic entoderm; *pr.c.*, proamniotic cavity.

several sections drawn in figure 30 is approximately indicated by the several crosses found to the left of the egg cylinder drawn in figure 29.

In A of figure 30, there may be observed a vacuolization of the protoplasm of the more peripherally placed cells of the ectoplacental cone, the vacuoles enclosing maternal blood cells. The more centrally placed cells of this ectoplacental cone show a tendency to concentric arrangement. Figures B and C present structural appearances nearly identical. The egg-cylinder is bounded by the thin layer of parietal or transitory ectoderm having scattered masses of nucleated protoplasm on its inner surface. This membrane of apparently homogeneous structure stains sharply in well fixed preparations and may be readily discerned. The cells of the visceral entoderm, somewhat taller in the section taken nearer the antimesometrial pole (C), present clearly the three zones to which attention has been drawn. The cells of the extraembryonic ectoderm bounding the mesometrial portion of the proamniotic cavity, are of cubic, short columnar, or polyhedral form disposed in single or double layer, presenting relatively lightly staining protoplasm. In D of figure 30, the cells forming the primary embryonic ectoderm are of distinct columnar shape, with relatively deeply staining protoplasm and nuclei arranged nearly in a single layer except for such as show mitotic phases. The cells of the primary embryonic entoderm are of a broad, pavement type for a greater part of the circumference, and may be contrasted with the cells of the visceral entoderm shown in B and C of the figure; the latter are absorptive cells, the former not. This series of figures, more especially B, C, and D, show clearly the absence of bilaterality in the egg-cylinders of the albino rat at this stage of development. The slight compression observed in this egg-cylinder, as shown in the figures, I regard as not of moment.

Fig. 31 Longitudinal sagittal section of egg-cylinder of the albino rat showing anlage of the mesoderm. $\times 200$. Rat No. 34, 8 days, 18 hours, after insemination; *ect.pl.*, ectoplacental cone or Träger; *p.ect.*, parietal or transitory ectoderm; *pr.emb.ect.*, primary embryonic ectoderm; *ex.ect.*, extraembryonic ectoderm; *pr.emb.ent.*, primary embryonic entoderm; *mes.*, mesoderm in anlage; *pr.c.*, proamniotic cavity; *v.ent.*, visceral entoderm.



Grosser has figured in his figures 68 and 114, an egg-cylinder of the albino rat which measures nearly 2 mm. in length. The age of this is given as $8\frac{1}{2}$ days. So far as may be determined from his figures, the preparation is not described in his text, the age, size, form, and structure of the egg cylinder shown in figure 29 and Grosser's figures 68 and 114, are very similar. In Grosser's figures, I see no evidence of his having differentiated between primary embryonic and extraembryonic ectoderm, while the reference letters for ectoderm and entoderm are reversed. Selenka's figure 31, plate 45, may be of a similar stage. This figure is, however, too diagrammatic to admit of close study. No difference is shown in the shape and structure of the cells bounding the two parts of the proamniotic cavity. Christiani's figure 39 may be of the same stage, but is too schematically drawn. Figure 4 of the article of Widakowich is of a slightly older stage and presents only a part of the egg-cylinder; it is recorded as about $6\frac{3}{4}$ days old. The stage under consideration is not figured by Widakowich, although his text description corresponds closely with what has been here presented.

The next stage and the one with which this communication is to be completed is one of importance since it is characterized by the anlage of the mesoderm. My own observations may be introduced with the consideration of an egg-cylinder, a section of which is presented in figure 31, rat No. 34, 8 days, 17 hours, after insemination. This was cut in the sagittal plane and measures 1.1 mm. by 0.2 mm., of which 0.4 mm. fall to the ectoplacental cone. This egg-cylinder is almost an exact duplicate, both in size and form, of that figured in figure 29 of the same age. In the egg-cylinder shown in figure 31, however, there may be observed, to one side, in the region of the junction of the primary embryonic and extraembryonic ectoderm, and between primary embryonic ectoderm and entoderm, a small group of cells which lie in close relation to the ectoderm and constitute early mesodermal cells. The sections of this series pass not exactly parallel to the mid-sagittal plane throughout the whole extent of the egg-cylinder; especially is this true of its antimesometrial portion, in the region of the primary embryonic

ectoderm. This portion in the section figured, passes a little to one side of the mid-sagittal plane. The two sections preceding the one figured enclose the mid-sagittal plane, and in them, the group of cells found between primary embryonic ectoderm and entoderm are in closer relation to the ectodermal layer and at all points distinctly separated from the entoderm. They are regarded as having wandered from the primary embryonic ectoderm to the place they occupy, a fact which is more easily ascertained in cross sections of a similar stage, as will appear from further discussion. From a study of very slightly older stages it can be determined that this region constitutes the primitive streak region of the future embryonic area. It is not my purpose at this time and in this communication to give especial consideration to the much discussed question of the origin of the mesoderm in Mammalia. In the rat, this question is complicated by the question of the anlage of the amniotic fold, which separates the proamniotic cavity into amniotic cavity proper and the ectoplacental cavity, the development of which will be considered in a projected contribution. In anticipation of this second publication, however, the following facts may here receive consideration. Widakowich presents in his figure 4, giving only the antimesometrial end of an egg-cylinder obtained the latter part of the 7th day, the anlage of the mesoderm as observed by him. This figure and my own figure 31 present almost identical relations, his figure showing only three mesodermal cells between primary embryonic ectoderm and entoderm. His own words concerning the anlage of the mesoderm in the albino rat, with which I find myself in full accord, except as to the age of the egg-cylinder, read as follows:

Das erste auftreten des Mesoderms beobachtete ich an Keimen vom Ende des 7 Tages. Die ersten Mesodermzellen liegen im Bereiche der vom mesometralen Ende des stärker färbbaren primären embryonalen Ectoderm gebildeten Falte. Es kommt hier eine ganz bestimmte Stelle in Betracht, die dort liegt, wo sich später das hintere Ende des Primitivstreifens befindet.

There is, however, wide divergence of the views of authors as concerns the anlage of the mesoderm in the rat and mouse.

Selenka, it would seem, in part at least, interpreted correctly the development of the mesoderm in the rat, although a stage showing its anlage was not observed. Duval believes that the mesoderm has origin from a thickened part of the entoderm, probably in the region of the anterior portion of the future embryonic area; the primitive streak was not recognized. Christiani's figures 45 and 47, transverse sections of the egg-cylinder from the eighth day, give correctly the relative position of the mesoderm with reference to the primitive streak; however, they show stages some little time after the anlage of the mesoderm. According to Robinson, in the early part of the eighth day the cavities of the epiblast (primary embryonic ectoderm) and of the trophoblast (extraembryonic ectoderm) meet and fuse to form a hollow cylinder, the proamniotic cavity. He states that "For a time the united cavities of the epiblast and trophoblast increase in size, together with the general growth of the ovum, and this increase continues until in the latter part of the eighth day the mesoblast appears around the margin of the epiblast where it is in apposition with the trophoblast." Robinson was able to differentiate between the primary embryonic ectoderm (epiblast) and the extraembryonic ectoderm (trophoblast) and his figure 14 (plate 23-24), though schematic, shows that he recognized the positions of the anlage of the mesoderm correctly, as also its derivation from the primary embryonic ectoderm. The observations of Melissinos, bearing on the anlage of the mesoderm have been critically reviewed by both Widakowich and Sobotta, and I am wholly in accord with their views when they state that no credence can be given these observations since it is clear that Melissinos has confused sagittal and frontal sections in such a way as to make his observations of no value. According to Melissinos, the mesoderm arises from the outer surface of the middle fold of the egg cylinder, in the region of its union with the antimesometrial ectodermal fold; it is certain that it does not arise from the part of the egg-cylinder that has differentiated from the primary embryonic ectoderm; but, if I interpret him correctly, from the extraembryonic portion of the ectoderm. That Melissinos did not

have before him the stages showing the anlage of the mesoderm seems clear. Sobotta's ('11) observations, mouse material, deserve fuller consideration. In interpreting his results, I am mindful of the fact that he was unable to locate the line of union between primary embryonic and extraembryonic ectoderm, as can readily be done in suitable rat material, as has previously been shown by Robinson and Widakowich, and to which attention has constantly been drawn in this communication. I am unable to state from personal observation whether in the white mouse these two types of ectoderm which form the lining of the proamniotic cavity, can be differentiated on ascertaining the right technical method. Sobotta's material seems well fixed. If not, it would seem to me difficult to determine definitely the exact place of origin of the mesodermal cells, whether extra-embryonic or embryonic. Sobotta recognized the anlage of the mesoderm in the mouse during the last hours of the seventh day or first hours of the eighth day. This is said to appear at the caudal end of the future embryo as a group of loosely arranged cells lying between the inner and outer layers of the egg-cylinder. At the place where the mesodermal cells arise from the inner layer of the egg-cylinder, there is developed a fold, recognized as the caudal amniotic fold ("Schwanzfalte des Amnios"). After discussing these observations at length, Sobotta concludes as follows:

Was die Deutung dieser frühen Stadien der Mesodermbildung in der Keimblase der Maus anlangt, so handelt es sich hier nicht um die Bildung des embryonalen Mesoderms, die erst mit der eigentlichen Gastrulation später einsetzt, sondern um Entstehung ausserembryonalen Mesoderms, besonder des Teils des mittleren Keimblattes, dass bei der Bildung der primären Eihäute, Amnios und Chorion in Betracht kommt und des den ausserembryonalen Teil der Leibeshöhle, das Exocoelom auskliedet, der Höhle, die eben Amnios und Chorion voneinander trennt. Es erfolgt also, um einen kurzen Ausdruck zu gebrauchen, die Bildung des Amniosmesoderms.

An embryonic anlage is said not to exist at this stage; this is recognized only after the development of the primitive streak. It is not my purpose to enter fully into a discussion of this important question in this communication. This would involve

consideration of older stages, and the making of a number of reconstructions, which it is not contemplated to consider now. It must suffice to state at this time that in the albino rat, as shown by Widakowich and here shown by me, it is possible to delineate clearly the primary embryonic ectoderm and to show that the first evidence of the mesoderm is found antimesometrial to the future amniotic fold and in the region of the future primitive streak; therefore is mesoderm which I would regard as peristomal mesoderm in the sense of C. Rabl, reference to which is made by Sobotta in his discussion of this question. It may be that the rat offers more suitable material for the elucidation of this question than is to be found in the mouse. In the albino rat, the anlage of the mesoderm is from the sagittal portion of the caudal region of the primary embryonic ectoderm, the caudal part of the future primitive streak and antimesometrial to the amniotic fold. Sobotta gives very favorable consideration to the observations of Widakowich, touching this question, which he regards as "Bei weitem die beste Darstellung des Gegenstandes." My own observations fully confirm those of Widakowich. These questions will receive fuller consideration in a later publication dealing with the embryology of the albino rat, carrying the development from the time of the anlage of the amniotic fold to the stage of embryo form, the material for which is at hand.

In figure 32 are shown cross-sections of the antimesometrial portion of three egg-cylinders in the region of the developing mesoderm. Sections drawn in A and B, were taken respectively from egg-cylinders obtained from the same uterus as was the one shown in sagittal section in figure 31, rat No. 34, 8 days, 17 hours, after insemination; C, from rat No. 41, 8 days, 16 hours, after insemination. It is very probable that the series from which A of this figure was drawn, is not cut in exactly the cross plane. A study of the series shows, however, that the deviation from this plane is not marked. The sections from which this figure was drawn pass a little below (antimesometrial) to the region of junction of the primary embryonic and extraembryonic ectoderm. To one side, the lower in the figure, the primary embryonic ectoderm shows a slight thickening and evidence of

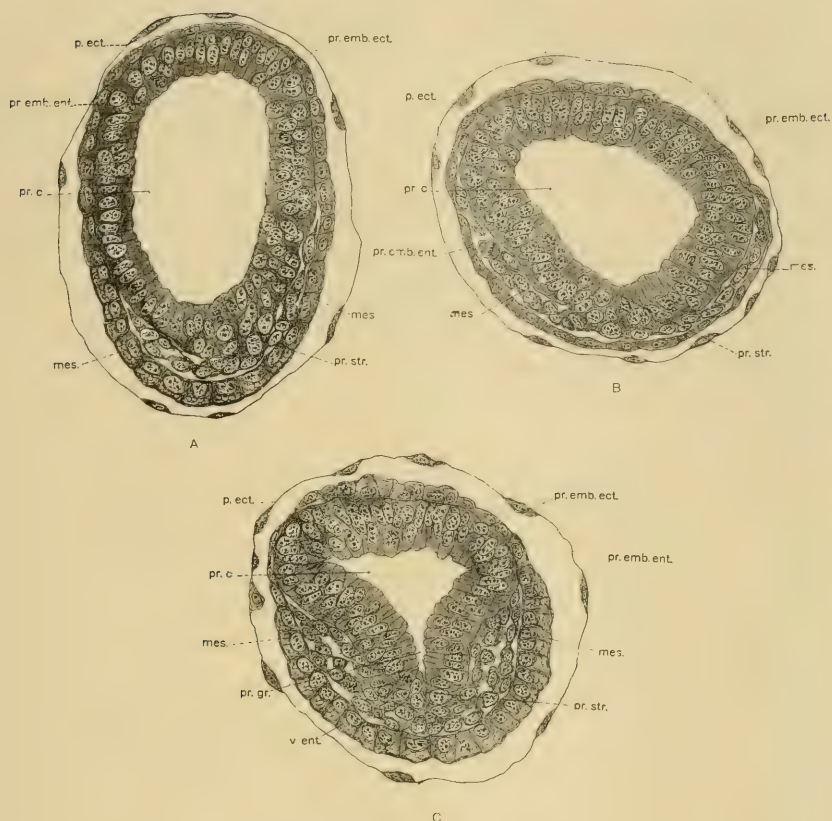


Fig. 32 Three cross sections from egg-cylinders of the albino rat, showing early stages in the development of the mesoderm. $\times 200$. A and B, rat No. 34, 8 days, 17 hours; C, rat No. 41, 8 days, 16 hours, after insemination.

These sections taken from three egg-cylinders are through the primary embryonic ectoderm, near its junction with the extraembryonic ectoderm, thus through the antimesometrial portion of the proamniotic cavity. A, early stage, in anlage of the mesoderm; B, anlage of the primitive streak and groove; C, well developed primitive streak and groove, with lateral wings of mesoderm; *pr.emb.ect.*, primary embryonic ectoderm; *pr.emb.ent.*, primary embryonic entoderm; *mes.*, mesoderm; *pr.str.*, primitive streak; *pr.gr.*, primitive groove; *p.ect.*, parietal or transitory ectoderm; *pr.c.*, proamniotic cavity.

cell proliferation. The cells of this region have not the form of tall columnar cells, such as seen in the greater part of the remaining primary embryonic ectoderm, but are of polyhedral

form and are continuous, in the mid sagittal plane, with cells that have wandered between the primary embryonic ectoderm and entoderm, cells regarded as constituting the mesoderm. In all of the sections of this series, so far as the mesoderm extends, this is distinctly separable from the entoderm, and is continuous with the primary embryonic ectoderm only along a narrow region of thickened primary embryonic ectoderm, situated in the mid-sagittal plane, and which may in this series be regarded as the anlage of the primitive streak. From the sides of this region of slightly thickened primary embryonic ectoderm, the extent of which is evidenced by the absence of an external limiting membrane, cells wander laterally to form the mesoderm. B, of figure 32, presents essentially the same appearance, although representing a slightly older stage. The sections of this series I regard as cut fairly well in a plane at right angles to the long axis of the respective egg-cylinder. The section taken for the sketch is situated a very little further away from the line of junction of the primary embryonic and extraembryonic ectoderm, than is the section the drawing of which is shown in A of this figure, as may be judged from the more uniformly pavement type of the entodermal cells. The triangular form of the proamniotic cavity is regarded as normal, and as indicating an early stage in the anlage of the primitive groove. In this figure, in its lower portion, the region of the primitive streak is readily discernible by reason of the fact that there is wanting here an external limiting membrane, and further by reason of the form of the cells and the form and relative position of their nuclei; certain of these cells indicating, both by their form and their position, the source and the direction of the wandering of the cells which constitute the anlage of the mesoderm. The wandering of the mesodermal cells between the primary embryonic ectoderm and entoderm, to form the lateral mesodermal wings, is clearly shown in this figure, especially to the left. The anti-mesometrial ends of the egg-cylinders, sections of which are shown in A and B of this figure, are as yet free from the invading mesoderm, as is also the part of the egg-cylinders lying opposite the region of the primitive streak, the upper portions of the

respective figures, these forming the region of the future anterior ends of the respective embryos. In C of figure 32 is shown a drawing of one of the sections of a series of cross-sections of an egg-cylinder taken from rat No. 41, 8 days, 16 hours, after insemination, presenting a stage in which the primitive groove may be definitely made out. This figure is not unlike figure 6 of the article of Widakowich, obtained from an egg-cylinder secured on the eighth day. Concerning this figure he states: "Das Ectoderm steht in direktem Zusammenhange mit zwei Mesodermzungen die gegen die der Primitivrinne gegenüberliegende Seite zu auswachsen." The section drawn in C of this figure is taken from the region very near the junction of the primary embryonic and the extraembryonic ectoderm, as may be observed from the character of the entodermal cells, in the lower part of the figure. The increase in the thickness of the mesodermal wings, the result, in part at least, of proliferation of mesodermal cells, as evidenced by the presence of mitotic figures, is clearly shown in this figure. The mesoderm is distinctly separable from the entoderm as also from the primary embryonic ectoderm except in the region of the primitive streak and groove. The growth of the mesoderm after its anlage has been correctly shown for the albino rat by Selenka, Robinson, and Widakowich; the latter especially giving excellent figures. His figure 5 is especially instructive. In this, he represents the appearances shown by two views of an isolated egg-cylinder, with the primitive groove in anlage, showing the lateral extensions of the mesoderm. Sobotta ('11) has given the best and most comprehensive account of the anlage and growth of the mesoderm in the mouse. An excellent cross-section of a mouse egg-cylinder in the primitive streak stage is presented in his figure 6, which presents very similar appearances to my C of figure 32. None of the figures of cross-sections of egg-cylinders included by me show the very beginning of the anlage of the mesoderm, though A of figure 32 approaches this very closely, as does also figure 31, presenting a sagittal section. The evidence at hand warrants the conclusion that in the albino rat, the mesoderm has its anlage in the caudal region of the primary embryonic

ectoderm, from a narrow zone of cells situated in the region of the future primitive streak. From this region there is an outwandering of cells which invade the potential cleft between primary embryonic ectoderm and entoderm, spreading laterally in wing-like sheets. This I would regard as prostomial mesoderm in the sense of C. Rabl. The anlage of the mesoderm in the albino rat, and the early stages of its lateral extension, with the anlage of the primitive streak and groove, falls to the latter part of the ninth day after insemination.

Beginning with the pronuclear stage, found at the end of the first day, 8 days are required for the completion of the process of segmentation, blastodermic vesicle formation and the formation of the primary germ layers—ectoderm, mesoderm, and entoderm—in all, 9 days out of a possible 21 to 23 days, the normal gestation period of the albino rat.

CONCLUSIONS

Early stages of mammalian development may readily be obtained from the albino rat (*Mus norvegicus albinus*). When care is exercised, mating may be observed and the age of the embryo, reckoned from the time of mating (insemination), determined with a fair degree of accuracy. Ovulations occur about the time of parturition and again 29 to 30 days post partem. This latter period is more favorable for obtaining insemination and semination, thus fertilized ova. The process of fertilization probably takes place during the latter half of the first day after insemination.

The pronuclear stage, a stage which extends through a period of perhaps 12 to 15 hours, in the middle phase, is observed at the end of the first day after insemination; the fertilized ova having wandered about one-fourth of the length of the oviduct by that time. Of the two pronuclei, the female pronucleus is slightly the larger. The two pronuclei lie near the center of the ovum, are distinctly membraned, and do not fuse prior to the formation of the first segmentation spindle.

The formation of the first segmentation spindle and the first segmentation occur during the early part of the second day after

insemination. The resulting 2-cell stage extends for a period of about 24 hours and is found in about the middle of the oviduct. The first two blastomeres are equivalent cells. One of these segments before the other, resulting in a 3-cell stage, present for each ovum for only a relatively short period.

The 4-cell stage is observed at the end of the third day after insemination. The ova have by this time traversed about nine-tenths of the length of the oviduct.

The 8-cell stage is observed the latter half of the fourth day after insemination and at the end of the fourth day the ova pass from the oviduct to the uterus in the 12-cell to 16-cell stage. The oolemma is lost usually in the 4-cell stage, the segmenting ova conforming in shape to the general form of that portion of the oviduct in which they are found.

Three successive segmentation stages, spaced at intervals of about 18 hours, resulting in 2-, 4-, and 8-cell stages occur during transit through the oviduct. During the fourth segmentation the ova pass from the oviducts to the uterine horns, at the end of the fourth day.

The mass increase of the ova during the first three segmentations is approximately from 0.15 c.mm. in the pronuclear stage to 0.18 c.mm. in the 8-cell stage. The slow rate of segmentation and the relatively small mass increase may be attributed to the relative scarcity of the embryotroph during transit through the oviducts.

During the early hours of the fifth day after insemination, all of the segmenting ova are found lying free in the lumen of the uterus, spaced about as in the later stages of development, the fifth series of segmentations having been completed by this time, the resulting morula masses having ovoid form, measuring approximately $80\ \mu$ by $50\ \mu$ and consisting of from 24 to 32 cells. The mechanism operative in spacing the ova in the uterine horns has not been determined.

The early stages of blastodermic vesicle formation are observed during the middle and latter half of the fifth day. The segmentation cavity begins as a single, irregularly crescentic space, eccentric in position, and arising between the cells of the morula.

By the end of the fifth day after insemination, all fertilized, normal ova are found in the blastodermic vesicle stage. One pole of each vesicle, its floor, consists of a relatively thick mass of cells, in which there is no differentiation in layers and no evidence of ectodermal and entodermal cells. The other pole of each vesicle, its roof, consists of a single layer of flattened cells, bordering the segmentation cavity.

During the sixth day, the blastodermic vesicles which still lie free in the lumen of the uterus, increase in size, partly as a result of extension of the roof cells, partly owing to rearrangement and flattening of the cells of the floor. This portion of the vesicle now presents the form of a concavo-convex disc, forming about one-sixth of the vesicle wall and consisting, as a rule, of three layers of cells, the inner of which is now differentiated to form the yolk entoderm.

During the seventh day after insemination the blastodermic vesicles become definitely oriented in a decidual crypt, the thicker portion, its floor, being directed toward the mesometrial border. The phenomenon of the "inversion of the germ layers" or "entypy of the germ layers" is initiated, the result of cell rearrangement and cell enlargement in the germinal disc, manifested as an outgrowth to form the ectoplacental cone or Träger and an ingrowth into the vesicle, the anlage of the egg-plug or egg-cylinder. In the egg-plug there is recognized a circumscribed, compact mass of cells, staining more deeply than surrounding cells, which constitute the ectodermal node, the anlage of the primary embryonic ectoderm of the future embryo. This ectodermal node, so far as it extends into the cavity of the blastodermic vesicle, is surrounded by yolk entoderm.

During the eighth day after insemination, the egg-cylinder comes in definite relation with the maternal decidua and receives as embryotroph maternal hemoglobin, partly through phagocytic action of the cells of the ectoplacental cone, partly through absorption of maternal hemoglobin by the cells of the entoderm, initiating a period of very active growth as evidenced by active mitosis. The egg-cylinder increases in length, and entypy is completed. A cavity develops in the ectodermal

node, the antimesometrial portion of the proamniotic cavity. A little later a second cavity develops in the extraembryonic ectoderm, the mesometrial portion of the proamniotic cavity, the two cavities fusing by the end of the eighth day to form a single proamniotic cavity, lined in its antimesometrial portion by primary embryonic ectoderm, and in its mesometrial portion by extraembryonic ectoderm, the two types of ectoderm forming a continuous layer with the line of junction readily distinguishable. No evidence of bilateral symmetry is at this stage observed in the egg-cylinder.

During the ninth day after insemination there is observed the anlage and the early developmental stage of the mesoderm and the anlage of the primitive streak and groove. The mesoderm has its anlage in the caudal portion of the primary embryonic ectoderm in the sagittal region and is of the nature of prostomial mesoderm, extending laterally in wing-like extensions between the ectoderm and entoderm.

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THE DEVELOPMENT OF THE ALBINO RAT,
MUS NORVEGICUS ALBINUS

II. ABNORMAL OVA; END OF THE FIRST TO THE END OF THE
NINTH DAY

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INTRODUCTION

In the course of my study of the normal development of the albino rat, from the end of the first to the end of the ninth day after insemination, as recorded in Part I of this series of contributions, there were encountered from time to time ova which appeared to deviate both in rate and type of development from what, as a result of extended study, came to be regarded as the normal developmental cycle of the albino rat. When taken collectively, the number of these abnormal ova is not large, although they embrace nearly all of the developmental stages studied. When taken singly, it may be stated that while it is comparatively easy to record the points of deviation from the normal, it must be admitted that the probable fate of the respective stages can only be conjectured. Nevertheless, a record of

the abnormal stages met with seems warranted, especially in view of the fact that the literature is very meager in its account of early stages of mammalian ova presenting abnormal development.

The excellent and comprehensive studies of Mall on pathologic human ova, extending over many years, may be interpreted as leading to the general conclusion that pathologic ova and monsters "are produced from normal eggs by conditions which either interfere with their nutrition or poison them." There is evidence to show that defective implantation, using the term in its broadest sense so as to include relation to the embryotroph or pabulum, is directly associated with abnormal development. Comparative experimental teratology so successfully followed by a number of European and American experimental embryologists warrants the conclusion that all of the abnormalities or malformations observed in the human embryo may be brought forth by the application of suitable mechanical interference or chemical solutions. Experimental teratology possesses the very great advantage of enabling the observer to follow the pathologic process from step to step, admitting more readily of their interpretation, than when single stages are obtained from nature. The evidence appears to be accumulating that the primary causes which produce pathologic ova lie not in the germ cells, but are rather to be sought in the environs of the germ cells in the course of their development.

I am cognizant of the fact that the interpretation of the chance findings of abnormal stages of mammalian ova is much more difficult than of abnormal ova produced experimentally. The fact, however, that nearly all of the abnormal ova observed by me in my albino rat material were found in tubes and uteri containing normal ova also, tubes and uteri which so far as observable appear in most instances to be normal, and the further fact that certain of the abnormal ova are of stages prior to what may be regarded as showing implantation, stages concerning which we possess no data as far as human ova are concerned, has led to the tentative conclusion that certain of the abnormal ova may be the resultant of abnormal germ cells, perhaps of an abnormality which may not show a structural expression.

It is my primary purpose to make records of the abnormal ova observed in the material at hand; and to follow these records with a brief consideration of the observations made. There is no literature dealing with the problem immediately at hand—abnormal rat ova. It is not my purpose at this time to enter into the extensive literature of comparative experimental teratology. This has been critically summarized relatively recently by O. and R. Hertwig, and by Mall, in his several contributions dealing with human pathologic ova.

HALF EMBRYOS IN MAMMALIA

The first preparation to which attention is called is one taken from the oviduct of rat No. 60, 1 day, 18 hours, after insemination. The two oviducts of this rat contained seven ova in the 2-cell stage, to one of which especial attention was drawn in Part I (page 271). As there recorded, in one of the 2-cell stages, the first two blastomeres were separated by an appreciable distance. There is loss of oolemma. The possibility of half embryos in Mammalia was suggested. The preparation under consideration is figured in figure 1, A and B. In A of this figure there is presented a portion of the wall of the oviduct, its epithelial lining and the immediately adjacent mucosa, including the fourth of a series of six sections ($10\ \mu$) passing through the two blastomeres. In this region, the cilia of the epithelium are clearly observable, as may be seen from the figure. In B of this figure there are sketched in approximately relative position the several sections of the series passing through the two blastomeres, the relative position of which, with reference to the walls of the tube, is shown in A of the figure. The six drawings were made from a well ribboned series; the slide was moved from section to section by means of a mechanical stage, and the perpendicular indicated on each drawing as made. The relative position of the several drawings, therefore, is quite correct. It may be observed that throughout the series the two blastomeres are separated by an appreciable space, and that one of the cells has rotated slightly on its axis. If these two blastomeres had remained in close apposition, they would present the appearance of a normal 2-cell stage

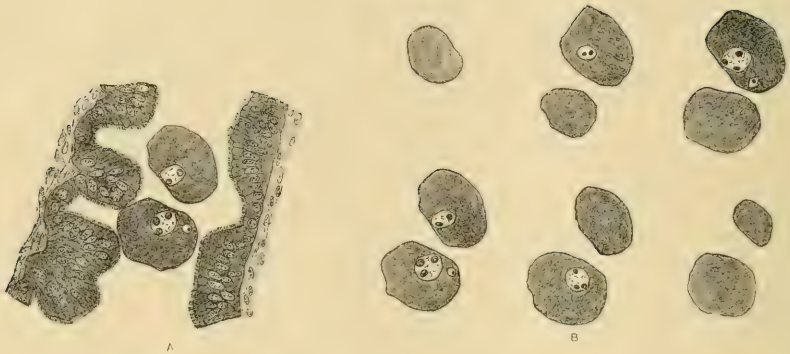


Fig 1 Oviduct and ovum of albino rat, in 2-cell stage, with first two blastomeres separated. Rat No. 60, 1 day, 18 hours, after the beginning of insemination. $\times 200$. A, epithelial wall of oviduct with adjacent mucosa, and the fourth of a series of six sections of the 2-cell stage with separated blastomeres, showing them in their relation to the epithelium. B, the series of six sections which pass through the separated blastomeres, the fourth of which is shown in A. The series reads from right to left.

as shown in B and C of figure 1, Part I. There is here clearly a separation of the first two blastomeres and not a close approximation of two unfertilized ova. In all of the unfertilized ova met with in the oviducts in the series at my disposal, these present the second maturation spindle and oolemma and are not to be confused with the blastomeres of the 2-cell stage, either as to size or structure. Both of the blastomeres in the preparation under consideration present normal protoplasmic structure, having a finely granular protoplasm. Their nuclei, as may be seen from the figures, are of normal size and structure. They present regular form, are distinctly membranated, have large chromatoid nucleoli, and chromatin scattered in fine granules and threads. However, attention needs to be drawn to the presence of two micro-nuclei, one in each of the two blastomeres, showing in the third and fourth section of the series respectively (B, fig. 1). These micro-nuclei are nearly free from chromatin, each presenting a small chromatoid nucleolus. They are not to be regarded as cell inclusions, as perhaps representing phagocytic leucocytes. It may be conjectured that they were formed by amitotic division, by budding and constriction from the parent

nuclei, perhaps indicating altered metabolism in the two blastomeres. I am inclined to think that both of these cells would have degenerated in the course of further development; however, their fate can only be guessed and not predicted. The possibility of their developing into half embryos is suggested. Half embryos developing as a result of a separation of the first two blastomeres has not been observed in the Mammalia, and an experimental test of the question is for the present not a probability.

As a result of experimental embryology it has been clearly shown that through mechanical interference polysomalous monsters may be produced from normal ova. The first two blastomeres are totipotent, as expressed by Driesch. Driesch was able to produce polysomalous forms by mechanical separation of the first two blastomeres in sea urchin eggs; Wilson, by separating through shaking of 2- and 4-cell stages in *Amphioxus*; O. Hertwig, Herlitzka and Spemann, by separating the first two cells in amphibian eggs; O. Schultze and others, by use of gravity and compression; and Loeb and others by use of chemical agents. By various means, then, when suitably applied and at the right time, hemiembryos have been produced by separating or potentially separating the first two blastomeres in certain forms. O. Hertwig states:

Bei den kleinen, mit geringen Mengen von Dotter ausgestatteten Eiern der Wirbeltiere sind spontan entstandene, das heisst, ohne experimentelle Eingriffe veranlasste Mehrfachbildungen ausserordentlich selten, bei manchen Klassen überhaupt noch nie beobachtet worden, dagegen sind sie relativ häufige Befunde bei manchen untersuchten Arten von Knochenfischen und Vögeln, besonders bei der Forelle und beim Hühnchen.

So far as I am aware, the possibility of hemiembryos in Mammalia has not been shown. In the albino rat, the oolemma may be lost as early as the 2-cell stage. In forms with early loss of oolemma, the separation of the two first blastomeres does not appear to me as an impossibility. The probable fate of separated mammalian blastomeres can only be conjectured, since it is manifestly impossible, for the present, to follow them in further development.

DEGENERATION AND DEATH OF OVA AT THE END OF
THE SEGMENTATION STAGES

In figure 2, A and B, are presented drawings of typical sections of two morula masses showing complete degeneration and death. The degenerated ovum shown in A, of this figure was obtained from rat No. 52, 4 days, 15 hours, after insemination. In all, eight normal ova were found in the uterus of this rat,

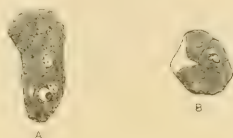


Fig 2 Ova of the albino rat in late segmentation stages, showing death and dissolution of the constituent cells. $\times 200$. A, rat No. 52, 4 days, 15 hours, after the beginning of insemination. B, rat No. 68, 4 days, 16 hours, after the beginning of the insemination. This figure shows an imperfectly developed morula with probable retention of oolemma.

these showing late morula stages and stages of early blastodermic vesicle formation, three of which were sketched and are shown in A, B, and C of figure 20, Part I. The degenerated ovum here under consideration lies in very close proximity to the normal blastodermic vesicle shown in C of figure 20, Part I. The shallow mucosal pits harboring the two ova are in contiguity. The two contiguous pits resemble each other very much; the mucosa underlying them is in every respect the same, indicating, it would seem, that to a certain stage in development—to the end of segmentation—the development of the degenerated ovum proceeded normally. The degenerated egg-mass measured approximately $80\ \mu$ by $50\ \mu$ by $40\ \mu$. In reaction to stains, it differs markedly from the adjacent normal vesicle. The staining is very pale; cell boundaries are indistinct or lost, and the nuclei scarcely retain any coloring matter. Scattered through the protoplasm are found small globular masses, perhaps of lipid character. Protoplasm and nuclei present evidences of cytolysis and chromatolysis, and have the appearance presented by necrotic tissue. Had normal development supervened, both ova

(the pathologic and the adjacent normal one) would in all probability have been enclosed within the same decidual crypt, a condition exceedingly rare, judging from the material at hand. Whether the very close proximity of these two ova bears causal relation to the death of one, by reason of the consequent lessening of the available pabulum or embryotroph, can only be conjectured. There is at this stage no question of faulty implantation, the ova, though presumably permanently lodged, lie free in the lumen of the uterus. Whether on the other hand, the death of this ovum was the result of some inherent nutritional deficit must also remain unanswered. However, this preparation may serve to show that ova of the albino rat, after reaching the uterine tube, and after apparently normal segmentation, may undergo death and dissolution, for reasons which are not structurally discernable.

B of figure 2, rat No. 68, 4 days, 16 hours, after insemination, is from the uterus of a rat containing four ova in early stages of blastodermic vesicle formation, three of which were sketched under D and E of figure 20, and the series of figure 21, Part I. The preparation here described lies free in the lumen of the uterus, and appears to represent an uncompleted segmentation, with cells and nuclei showing cytolysis and chromatolysis. The mass is surrounded by a thin membrane regarded as an oolemma. Normally the oolemma of the segmenting ova of the albino rat is lost in the 4-cell stage, now and again in the 2-cell stage. Whether the retention of the oolemma may be brought in causal relation to the death and dissolution of the enclosed cells is problematic. That such causal relation may exist for the ova of the albino rat, appears to me as not impossible. This degenerated egg-mass presents the only instance of the late retention of the oolemma in the albino rat material at my disposal.

INCOMPLETE OR RETARDED SEGMENTATION

The blastodermic vesicles presented in figures 3 and 4 have been interpreted as showing incomplete or retarded division of certain of the cells of early stage morula masses. The probable fate of such blastodermic vesicles in further development cannot

be projected with any degree of certainty. The most characteristic vesicle showing this phenomenon is presented in figure 3, and is taken from rat No. 53, 5 days after insemination, the uterus of which contained seven blastodermic vesicles showing early stages of development, four of which are reproduced in figure 22, Part I. In A and B of figure 3 are reproduced two consecutive sections of a series of five sections of 10 μ thickness, including this ovum. In the lower part of this ovum there is found a small segmentation cavity, bounded by cells which present normal appearances. The roof of this vesicle is slightly

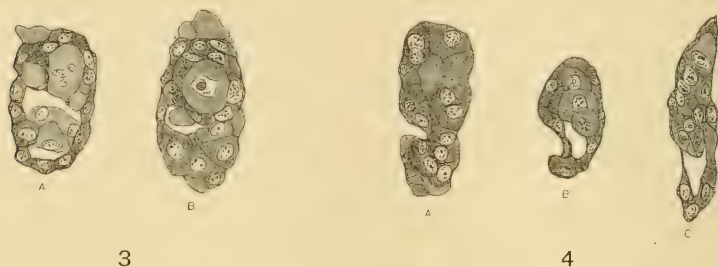


Fig. 3 Early stages of the blastodermic vesicle of the albino rat, presenting evidence of irregular or retarded segmentation. $\times 200$. Rat No. 53, 5 days after the beginning of insemination.

Fig. 4 Three ova of the albino rat, showing early blastodermic vesicle stages, in each of which certain of the cells suggest irregular or retarded segmentation. $\times 200$. A, rat No. 64, 4 days, 14 hours, after the beginning of insemination. B, rat No. 68, 4 days, 15 hours, after the beginning of insemination. C, rat No. 54, 6 days, 16 hours, after the beginning of insemination.

folded and compressed, as a consequence of which the roof wall in the sections figured is presented in part as seen in surface view. In the floor of this vesicle there is to be observed, surrounded by other smaller cells, one large cell, of nearly spherical shape, having a diameter which is three or four times as great as that of the majority of the surrounding cells. The protoplasm of this large cell stains less deeply than does that of the majority of the other cells constituting the floor of the vesicle. Its nucleus is relatively large and slightly lobulated, so much so that in the section of it shown in A of this figure, in the optical section sketched, the nucleus appears as three separate nuclei, in reality,

lobules of the same nucleus. In A of this figure there is shown to the lower left of the large cell another relatively large cell, enclosing a globular inclusion, which stained faintly, and the nature of which was not fully determined. In the upper part of each of the two figures are seen cells which show cytolysis and loss of nuclei; regarded as degenerating cells. When compared with the normal blastodermic vesicles obtained from the same uterus, the ovum here described presents a unique appearance, and was readily recognized as showing development and structure which deviated from the normal. At this stage of development, the blastodermic vesicles of the albino rat are still found lying free in the lumen of the uterus, showing no structural relation to the uterine mucosa. This vesicle has been interpreted as showing irregular or retarded segmentation. It is conjectured that one of the cells, perhaps of the 8-cell stage, did not undergo further cleavage. The large cell presents an appearance evidencing beginning stages of degeneration, and in further development, would probably have undergone dissolution. The majority of the smaller cells of the roof appear as if normal, as do also the cells of the floor, certain of the smaller cells of the floor presenting mitoses as evidence of further proliferation.

In figure 4, A, B, and C, there are presented typical sections of three ova of the albino rat showing what has been regarded as irregular segmentation. A of this figure represents an ovum taken from rat No. 64, 4 days, 14 hours, after insemination, in the uterus of which there were found five normal ova showing early stages of blastodermic vesicle formation, four of which are cut longitudinally, one in a series of cross-sections. In each of the four longitudinally cut series the floor of the respective vesicles is markedly folded, owing to fixation contractions; therefore, none were sketched as normal stages. In appearance, they resemble closely the vesicles sketched under C, D, and E of figure 20, Part I. In the pathologic ovum, shown in A of figure 4, there is no evidence of segmentation cavity formation. However, the ovum cannot be regarded as presenting a late morula stage such as is figured in A of figure 20, Part I, since it shows distinct departure from the normal. The marked constriction

seen to the lower left of the figure passes through the series of four $10\ \mu$ sections including this ovum, and in part separates a portion composed of relatively small cells from a larger portion composed of larger cells. The rate of segmentation of certain of the cells composing the upper larger portion of this cell mass appears to have been retarded, thus retarding the development of the whole mass. This pathologic ovum rests normally in a shallow pit of the mucosa, very similar in form and structure to the shallow pit lodging the five normal vesicles found in this uterus.

The ovum shown in B of figure 4 was obtained from the uterus of rat No. 68, 4 days, 16 hours, after insemination. with four normal vesicles showing early stages of blastodermic vesicle formation. From this uterus was also taken the completely degenerated cell mass with persistent oolemma shown in B of figure 2. This vesicle on superficial observation does not appear to depart markedly from the normal appearance for this stage. In form and size it corresponds closely to the normal ova taken from this uterus. The segmentation cavity seems to have developed normally. The slight folding of the roof seen to the left of the figure is accidental, due to fixation shrinkage, and is very similar to folding of the roof to be observed in many of the normal preparations of the series. In the floor of the vesicle there may be observed three relatively large cells, partly enclosed by smaller cells of a size comparable to that of the cells forming the floor of the normal blastodermic vesicles of this stage of development. The three relatively large cells, clearly distinguished in the figure, are interpreted as showing a retarded segmentation. So far as may be determined, their protoplasm and nuclei present normal structure, the lowest of the three cells showing an early mitotic phase. I am inclined to the opinion that this ovum would have continued in development, perhaps in later stages showing distinct arrest in development. This hypothesis seems warranted on the basis of the study of a vesicle shown in C of figure 4, taken from rat No. 54, 6 days, 16 hours, after insemination. Normal stages for the albino rat, taken about the middle of the seventh day after insemination, are shown in figure

24, Part I. Reference to this figure may serve to show that during the early hours of the seventh day after insemination, the phenomenon of inversion or entypy of the germ layers is initiated in the albino rat. The ova are, on reaching this stage of development, enclosed within a well differentiated decidual crypt which communicates as yet freely with the lumen of the uterus. These crypts present a continuous lining of uterine epithelium; the contained ova are thus not as yet in direct relation with the maternal decidua. In the normal blastodermic vesicle of this stage, the ectoplacental cone is in anlage, and in the cell mass which extends into the cavity of the vesicle—the egg-plug or egg-cylinder—there is evident a clearly circumscribed nodule of cells, which has been designated the ectodermal node and recognized as the anlage of the primary embryonic ectoderm; this node is in part surrounded by the yolk entoderm. In the uterus of rat No. 54, there are contained nine blastodermic vesicles, one of which is sketched in C of figure 24, Part I. Not nearly all of these vesicles are so favorably cut as that shown in this figure, the majority being cut in a plane which is oblique to the long axis of the vesicle. However, in all of them the ectoplacental cone and the ectodermal node may be determined except in the one shown in C of figure 4. This vesicle was obtained from a series of sections passing at right angles to the plane of the mesometrium. It lies free in a deep decidual crypt and passes through six sections of 10 μ thickness; thus is compressed from side to side. This vesicle is distinctly smaller than the normal ones taken from this series, especially so as concerns its cavity. An ectoplacental cone is not clearly differentiated, and it is not possible to determine an ectodermal node, nor is it clear that the yolk entoderm has differentiated. In the cell mass from which ectoplacental cone and ectodermal node should have developed, the upper portion of this figure, there are evident, in the sections figured, four relatively large cells with relatively large nuclei, cells which have been interpreted as evidencing retarded segmentation with consequent retardation in the normal differentiation of the vesicle. On tracing this vesicle through the series of six sections it would seem that the direction of section is favor-

able. The uterine mucosa appears to have reacted normally; the decidual crypt in which this vesicle is lodged presenting normal size and form, and the surrounding decidua normal structure. The vesicle itself is retracted from the uterine epithelium, intact throughout the crypt, thus, does not appear to have attained the normal adhesions observed in normal vesicles of this stage. The four ova depicted in figures 3 and 4, appear to present a distinctive type of abnormal development, a type which is interpreted as showing retarded segmentation in certain of the cells of the 8-cell and perhaps 16-cell stage. All are found in



Fig. 5 Four consecutive sections of the ovum of the albino rat showing abnormal development of the segmentation cavity $\times 200$. Rat No. 46, 6 days, 14 hours, after insemination.

uteri containing normal stages. The appearances presented, if correctly interpreted, speak in favor of a structural or metabolic defect inherent in the cells themselves and not primarily dependent on environment, pabulum, or embryotroph.

ABNORMAL SEGMENTATION CAVITY FORMATION

The following three ova have been grouped as showing irregularity in the formation of the segmentation cavity.

In figure 5 are reproduced four consecutive sections passing through an abnormal ovum obtained from rat No. 46, 6 days, 14 hours, after insemination. There were obtained from the uterus

of this rat ten blastodermic vesicles, two of which are reproduced in A and B of figure 24, Part I, as showing typically early stages of the anlage of the ectoplacental cone and entypy of the germ layers. The ovum shown in figure 5 is found in a decidual crypt which is in very close proximity to the one containing the vesicle figured under B of figure 24, Part I, the two crypts being separated by a distance of approximately 1.3 mm., while the distance between decidual crypts is normally 1 cm. to 1.5 cm. The decidual crypt lodging the abnormal ovum presents a normal appearance, resembling very closely in form, depth and structure

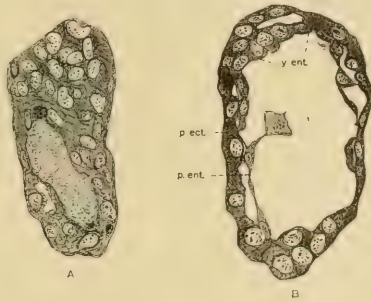


Fig. 6 Two ova of the albino rat, interpreted as evidencing retarded or irregular formation of the segmentation cavity. $\times 200$. A, rat No. 90, 6 days, 17 hours, after the beginning of insemination. B, rat No. 90, 6 days, 17 hours, after the beginning of insemination. *p.ect.*, parietal or transitory ectoderm; *y.ent.*, yolk entoderm; *p.ent.*, parietal entoderm.

of the surrounding decidua, the crypt and decidua enclosing the adjacent normal vesicle figured in B of figure 24, Part I. The abnormal ovum in question appeared to have proceeded normally in segmentation, its constituent cells being of about the size and structure of the cells of normal vesicles taken the early part of the seventh day after insemination. The cell-mass encloses a relatively small cavity which may be regarded as an abnormally placed segmentation cavity, in that its position is not eccentric, and that it is surrounded on all sides by more than one layer of cells. There is thus no differentiation of floor and roof as in normal blastodermic vesicles, and no development of ectoplacental cone and egg-cylinder as in the other ova obtained from

this uterus. I am for the present unable to offer any plausible explanation or give reasons for such abnormal development of the segmentation cavity. The fate of such a structure may perhaps be conjectured from a study of the abnormal ovum shown in A of figure 6, interpreted as showing a similar abnormality, but obtained in early stages of degeneration. This ovum and that shown in B of the same figure was obtained from the uterus of rat No. 90, 6 days, 17 hours, after insemination. In the uterus of this rat there are found six ova, only one of which was developed to a stage comparable to that shown in figure 24 (Part I) of about the same age. Three other vesicles present a slightly younger stage and may be compared with vesicles shown in D and E of figure 23, Part I. None of these four vesicles is favorably cut, but so far as may be determined, are of normal structure for the respective stages represented. A of figure 6 is also cut slightly obliquely, not sufficiently so, however, to make difficult its interpretation. The figure drawn is that of the third of a series of seven sections having $10\ \mu$ thickness, and depicts what is regarded as representing an ovum with abnormal segmentation cavity formation. In this ovum, the segmentation cavity is slightly more eccentric than is that shown in figure 5, and contains a granular detritus which in the preparations is distinctly stained with Congo red. The roof of this vesicle is composed almost throughout of more than one layer of cells. There is no differentiation of ectoplacental cone and ectodermal node, nor of yolk entoderm. Two cells regarded as phagocytic leucocytes, staining much more deeply in Congo red than do the cells of the ovum, have, in the section figured, penetrated the egg-mass, indicating early degenerative changes.

The vesicle shown in B of figure 6, obtained from the same rat, is favorably cut, and is readily followed through the series. The structural appearance presented by this vesicle is not explained by supposing it due to very oblique plane of section of a normal vesicle, a plane of section which might include the roof of the vesicle while avoiding its floor. The vesicle is abnormal in that it presents a want of development of the thickened germ disc, and a hyperdevelopment of the yolk entoderm. In none of the

sections of the series which includes this vesicle, which is cut in very favorable longitudinal direction, and is thus readily oriented with reference to mesometrial and antimesometrial portion, is there seen any thickening of the outer layer of cells, to form the part known as the floor of the vesicle, which at this stage of development is uniformly directed toward the mesometrial border. In A and B of figure 23, Part I, are shown vesicles with which the ovum here discussed may be compared. In the preparation under discussion, the yolk and parietal entoderm form almost a continuous layer, one of the detached cells showing a mitotic phase. In the normal vesicles of this stage of development the parietal entoderm is represented by a few scattered cells, as may be observed by a study of the figures to which reference is above made. Whether this vesicle is to be regarded as showing a later stage of an ovum in which there was irregularity in the formation of the segmentation cavity, I must for the present, leave as problematic. It has occurred to me that by enlargement of the segmentation cavity of an ovum such as shown in figure 5, with centrally placed segmentation cavity, there might result in further development the formation of a vesicle such as shown in B of figure 6.

It is freely admitted that the deductions here made, relative to irregularity in the formation of segmentation cavity, are not supported by conclusive evidence. It has seemed to me, however, that the interpretations given to the appearances presented are less open to criticism than others that might be suggested. These abnormal ova also suggest an inherent defect in the ova, leading to abnormal development, rather than abnormal development resulting from defective environment.

DEGENERATION OF OVA AS RESULT OF PATHOLOGIC UTERINE MUCOSA

In figure 7 are reproduced two ova which seem to me to show the primary stages of degeneration owing to pathologic condition of the uterine mucosa. Vesicle A was taken from the uterus of rat No. 91, 5 days, 16 hours, after insemination. In the uterus of this rat there were found only two ova. Vesicle B was taken

from rat No. 104, 6 days after insemination. In the uterus of this rat there were found six ova. In both of these rats, the ova present essentially the same stage of development, comparable to that shown in A and B of figure 23, Part I. As may be observed from the text of Part I (page 301) the stages obtained at the end of the sixth day and early hours of the seventh day, were found very difficult to fix. At this stage the ovum consists of a relatively large, thin walled vesicle, very prone to fixation shrinkage. All of the ova or vesicles obtained from rats Nos. 91 and 104, are very badly folded in their roof portion. Those shown

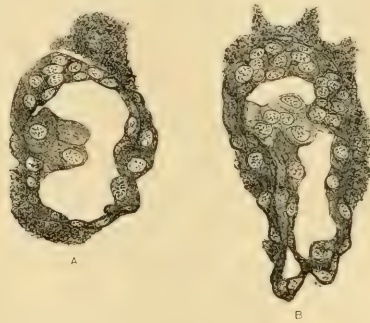


Fig. 7 Two ova of the albino rat partly surrounded by maternal blood with many phagocytic leucocytes. The folding of the roof of the vesicles is due to fixation shrinkage. $\times 200$. A, rat No. 91, 5 days, 16 hours, after the beginning of insemination. B, rat No. 104, 6 days after the beginning of insemination.

in A and B, figure 7, are representative. This folding, a result of imperfect fixation, is present in all of the vesicles of this stage, even though the respective vesicles present normal structure. The ova here figured may be regarded as having fairly normal structure, both as to rate of development and as to arrangement, form, and structure of constituent cells. All of the eight vesicles obtained from these two rats (No. 91, 2 ova; No. 104, 6 ova) are in part surrounded by exudated maternal blood, containing numerous leucocytes. Small masses of blood with leucocytes are found here and there in different parts of the uterine lumen of both rats, lodged in mucosal folds other than the characteristic decidual crypts enclosing the respective ova. These

decidual crypts are relatively shallow when compared with those of normal uteri of similar stages with normal ova. The uterine mucosa of the two rats under discussion does not appear to have reacted in a normal manner. In these preparations, attention is especially drawn to the presence of maternal blood with numerous phagocytic leucocytes found in relation with the ova, a condition never observed in normal development of ova and uterine mucosa. In A and B, figure 7, the red and white blood cells with granular detritus may be observed as found in relation with the respective vesicles, these presenting essentially the same appearances as do the other six ova obtained from these two rats; the one figured having been more favorably cut than any of the others. The appearances presented in these two rats are interpreted as showing a probable degeneration of the eight ova, and probably complete dissolution and removal. The vesicles appear to have developed normally to the stage at which they were obtained. As a result, however, of pathologic condition of the uterine mucosa, maternal blood, especially leucocytes, have entered the lumen of the uterus, the leucocytes being destined to play the rôle of phagocytes. In normal development of the albino rat, maternal blood does not enter the lumen of the uterus—decidual crypts—until after the uterine epithelium has become detached from the mucosa of the wall of the decidual crypt, in the region of lodgment of the enclosed ovum. Normally, very few leucocytes are met with in the lumen of the uterus, even in later stages of development, stages in which maternal red blood cells are met with in the decidual crypts. After experience had accumulated, uterine tubes supposed to contain developmental stages aging from the fourth to the sixth day, which on examination revealed blood and especially leucocytes in the lumen of the uterus, were regarded as not favorable specimens for finding ova. In a number of such uteri, cut completely in serial sections, no ova were found. It is possible that, owing to phagocytic action of the leucocytes present, the ova may have been completely removed prior to killing and fixing the tissues. In such condition, it would seem to me as pertinent to speak of faulty implantation, due to abnormal uterine mucosa. It seems to me signifi-

cant that in the two rats in which the pathologic condition affects primarily the maternal tissue, the uterine mucosa, all of the contained ova are prone to degeneration. In the abnormal ova previously described, for which it was suggested that the causes for the abnormality were to be sought in the ova themselves, in the great majority of instances, only one abnormal ovum was found in each uterus along with a variable number of ova which are to be regarded as normal for the respective stage.

IMPERFECT DEVELOPMENT OF THE ECTODERMAL VESICLE

The series contains two ova, very favorably cut, ova in which the ectodermal vesicle with the antimesometrial portion of the proamniotic cavity does not seem to have developed normally. Stages showing the differentiation of the egg-cylinder, the formation of the ectodermal vesicle with the antimesometrial portion of the proamniotic cavity, the formation of the mesometrial portion of the proamniotic cavity in the extraembryonic ectoderm, the union of the two primary proamniotic cavities to form a single space, are clearly shown in figures 26 and 27, Part I, in the series of closely approximated stages there portrayed. From a study of these figures, it will be observed that the antimesometrial portion of the proamniotic cavity develops within the ectodermal node before the mesometrial portion of this cavity develops in the extraembryonic ectodermal portion of the egg-cylinder. In the egg-cylinder shown in figure 8, rat No. 94, 8 days after the beginning of insemination, such is not the case. In the uterus of this rat there were found seven egg-cylinders, one of which, very favorably cut, is shown in C, figure 27, Part I. The other egg-cylinders obtained from this uterus, except the abnormally developed one to be discussed, though not favorably cut, present essentially the same form and structure as that figured under C of the figure above referred to. The egg-cylinder portrayed in figure 8 compares in size and form with those regarded as normal and taken from the same uterus. For the greater part it presents normal structure and normal relations of cells. The ectoplacental cone, only in part included in the figure, and the parietal

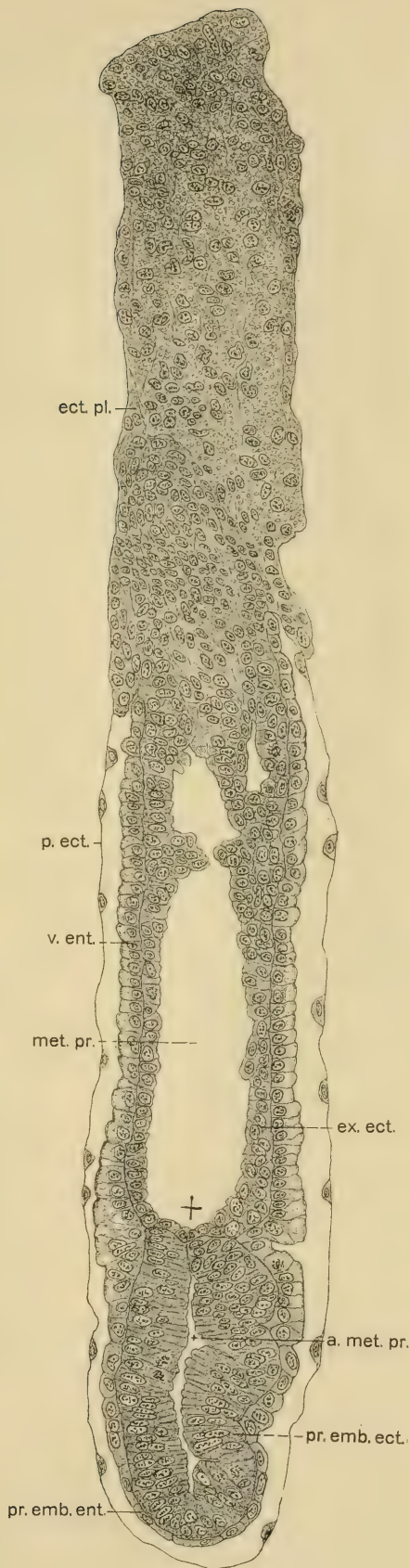


Fig. 8 Egg-cylinder of albino rat showing retarded development of ectodermal node and of the formation of the antimesometrial portion of the proamniotic cavity. $\times 200$. Rat No. 94, 8 days after the beginning of insemination. *ect.pl.*, ectoplacental cone or Träger; *v.ent.*, visceral entoderm; *met.pr.*, mesometrial portion of the proamniotic cavity; *p.ect.*, parietal or transitory ectoderm; *pr.emb.ent.*, primary embryonic entoderm; *ect.n.*, ectodermal node; *a.met.pr.*, imperfectly developed antimesometrial portion of proamniotic cavity; *ex.ect.*, extraembryonic ectoderm.

ectoderm, in structure and relation to decidual crypt, are to be regarded as of normal development. The visceral entoderm, surrounding the extraembryonic ectodermal portion of the egg-cylinder, is of normal structure, showing the three zones evidenc-

ing its absorptive function. The extraembryonic ectoderm, enclosing the mesometrial portion of the proamniotic cavity, presents normal structure and relations of cells. The only abnormality observed is in the region of the ectodermal node, the anlage of the ectodermal vesicle with the enclosed antimesometrial portion of the proamniotic cavity. With this stage of development of the egg-cylinder (see figs. 26 and 27, Part I) the ectodermal node presents a well formed cavity, surrounded by the cells of the primary embryonic ectoderm, radially arranged. In the egg-cylinder under discussion (fig. 8) there is distinctly a retardation in the development of the ectodermal vesicle with full differentiation of the primary embryonic ectoderm. An imperfectly developed antimesometrial portion of the proamniotic cavity is evident. This small cavity, indistinctly bounded, extends obliquely through several sections of the ectodermal node, and contains amorphous granular detritus, which in the preparations is stained by Congo red. The cells destined to form the primary embryonic ectoderm show no definite arrangement, especially as concerns the more centrally placed cells of the node. Since the primary embryonic ectoderm is the anlage for the ectoderm of the embryo, an arrest in its differentiation would of necessity profoundly affect further development of the embryo. Antimesometrial to the ectodermal node (just above it in the figure) there is found a small vesicle the walls of which are not distinctly delimited and composed of extraembryonic ectodermal cells, surrounding a small, completely bounded cavity. I am not prepared to say whether this small vesicle is to be regarded as developing from cells of the extraembryonic ectoderm, or from a displaced, accessory ectodermal node, in which a discrete portion of the proamniotic cavity has developed. If the latter, the possibility of a double anlage for the embryonic ectoderm is to be considered. My interpretation of this egg-cylinder as showing a retardation of the development of the ectodermal node and differentiation of the primary embryonic ectoderm, is confirmed from a study of a slightly older stage showing essentially the same condition. This ovum is presented in figure 9, and is taken from rat No. 41, 8 days, 16 hours, after the

Fig. 9 Egg-cylinder of albino rat, in which the antimesometrial and mesometrial portions of the proamniotic cavity have failed to unite to form a single or definite proamniotic cavity. $\times 200$. Rat No. 41, 8 days, 16 hours, after the beginning of insemination. *ect.pl.*, ectoplacental cone or Träger; *p.ect.*, parietal or transitory ectoderm; *v.ent.*, visceral entoderm; *met.pr.*, mesometrial portion of the proamniotic cavity; *ex.ect.*, extraembryonic ectoderm; *a.met.pr.*, antimesometrial portion of the proamniotic cavity; *pr.emb.ect.*, primary embryonic ectoderm; +, region at which, in normal development, by the end of the eighth and beginning of the ninth day, the two portions of the proamniotic cavity would have united to form a single space, the definite proamniotic cavity.



beginning of insemination. The uterus of this rat contains eight egg-cylinders, all of which, except the one here figured, show normal structure, though presenting quite different stages of development. One of these, cut serially in cross-section, is figured in C, figure 32, Part I, as showing anlage of mesoderm with primitive streak and groove. Two of the other egg-cylinders show the anlage of the mesoderm, two others show late pre-mesoderm stages of the egg-cylinder, the remaining egg-cylinders are less fully developed, one showing a development which may be compared to B of figure 26, Part I, thus a much younger stage. By the end of the eighth day and with the early hours of the ninth day after the beginning of insemination in the albino rat, the two parts of the proamniotic cavity, which develop discretely, have joined to form a single space (C, fig. 27, Part I). The egg-cylinder shown in figure 9, presents normal development in all parts, except that there is as yet no union of the two parts of the proamniotic cavity. This egg-cylinder is most favorably cut, in longitudinal direction; the plane of section being almost parallel to the mid-sagittal plane. This egg-cylinder, therefore, is easily followed through the several sections of the series into which it was cut. The irregularity of outline of the ectodermal vesicle, lower right of figure, it is believed, is not due to fixation shrinkage. Judging from size and structural differentiation of this egg-cylinder, union of the antimesometrial and mesometrial portions of the proamniotic cavity should have been completed before this stage of development was reached, with the primary embryonic ectoderm and the extraembryonic ectoderm forming a continuous layer, as shown in figure 29, Part I. The folding of the wall of the antimesometrial portion of the egg-cylinder, lower right of figure, evident in nearly all of the sections of the series, is regarded as indicating an abnormal growth of the primary embryonic ectodermal cells composing the wall of the ectodermal vesicle, as a result of retarded extension of the antimesometrial portion of the proamniotic cavity, perhaps an adjustment to meet the altered mechanical stress resulting from abnormal development. The condition here seen, it would seem, is foreshadowed in the egg-cylinder shown in figure 8.

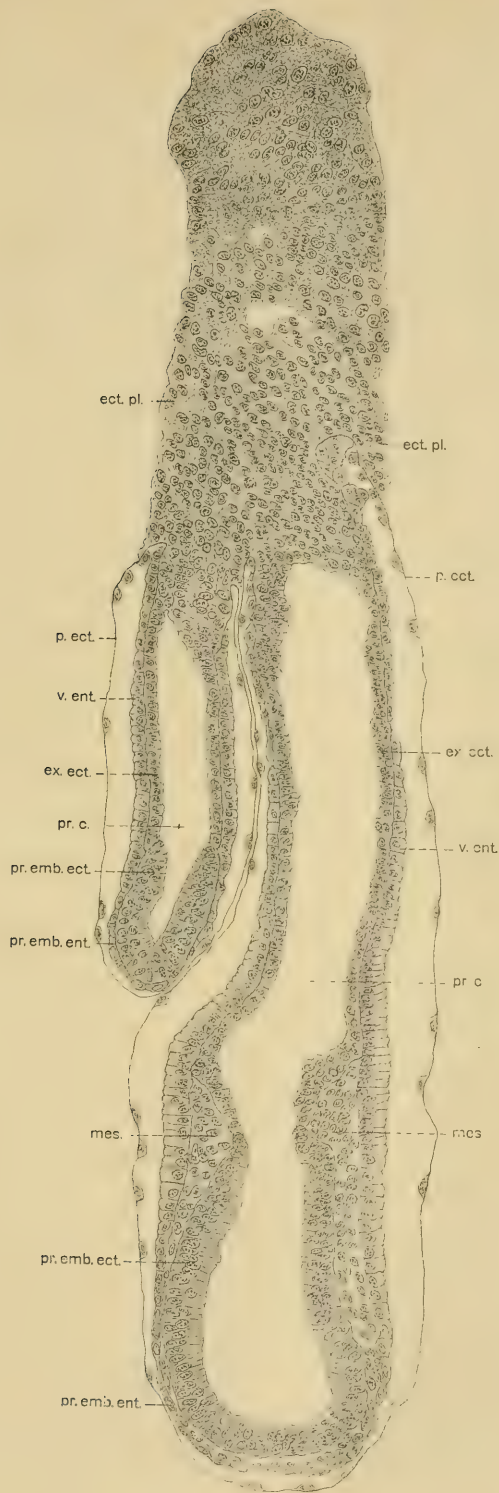


Fig. 10 Two egg cylinders of the albino rat found within the same decidual crypt, with in part common ectoplacental cone. $\times 150$. Rat No. 87, 9 days after the beginning of insemination. *ect.pl.*, ectoplacental cone or Träger; *p.ect.*, parietal or transitory ectoderm; *v.ent.*, visceral entoderm; *ex.ect.*, extra-embryonic ectoderm; *pr.c.*, proamniotic cavity; *pr.emb.ect.*, primary embryonic ectoderm; *pr.emb.ent.*, primary embryonic entoderm; *mes.*, mesoderm.

The causes operative in this retardation of development and differentiation of the ectodermal vesicle and primary embryonic ectoderm, I have been unable to determine. They would appear to be inherent in the egg-cylinder, since ectoplacental cone and visceral entoderm, so far as may be determined from a study of sections, appear to have functioned normally, in furnishing the necessary embryotroph in the form of maternal hemoglobin, as is normal for egg-cylinders of the albino rat of this stage of development:

TWO EGG-CYLINDERS IN ONE DECIDUAL CRYPT

The ova portrayed in figure 10 present a condition which must be regarded as exceedingly rare, since it represents the only instance of this condition observed in the extended series of preparations of the various stages of the development of the albino rat from the end of the first to the end of the ninth day after insemination, in my possession. This preparation is from rat No. 87, 9 days after the beginning of insemination. The uterus of this rat contained, other than the preparation here considered, six egg-cylinders of normal development, all showing a stage which is slightly older than that shown in figure 31, Part I, in that the mesoderm shows further development than is shown in that figure. In the preparation here figured there are found two egg-cylinders enclosed within the same decidual crypt. This figure, which is drawn by combining the drawings made from two sections, is reproduced at a magnification of 150 diameters, while all of the other figures portraying sections of ova, both in Part I and in Part II of this communication, are reproduced at a magnification of 200 diameters. This should be borne in mind when comparing this figure with the others. In figure 10, the lower portion of the large egg-cylinder to the level of the lower end of the smaller one was drawn from one section, while the remainder of the figure was drawn from the fourth following one. The adjustment was made by overlapping in the camera lucida drawing ($\times 600$) the sharp mesometrial border of the primary embryonic ectoderm of the larger egg-cylinder. Scarcely any

adjustment was found necessary, none of the right wall of the larger egg-cylinder, and only very slightly so of its left wall. The slight deviation from the longitudinal axis of the larger egg-cylinder made the procedure desirable. It is thought that the figure as presented gives correctly the size of the respective egg-cylinders, and in all essentials, their relations; the greater part of the figure having been drawn from one section. Both of the egg-cylinders reveal normal structure for the stages of development attained. The larger one is cut in the coronal plane, as is readily determined by the distribution of the mesoderm, one side representing a mirror picture of the other. The direction of section in the smaller egg-cylinder, except that it is longitudinal, is not to be determined, since before the anlage of the mesoderm, a bilateral symmetry cannot be recognized in sections. Since these two egg-cylinders are in all essentials of normal form and structure, and since their structure is clearly brought out in the figure, an extended description of them at this place seems uncalled for. For respective stages the reader is referred to Part I. Attention may be drawn, however, to the fact that the visceral entoderm on the contiguous surfaces of the two egg-cylinders is less fully differentiated, and shows less absorption of the maternal hemoglobin than is seen on the exposed or free surfaces, this, no doubt, for mechanical reasons. Further, that in the region where the two egg-cylinders are in contact, the parietal ectoderm of each can be traced as a distinct layer to the bases of the respective ectoplacental cones, showing that each developed from a separate ovum. The ectoplacental cones are for a short distance distinct. In tracing the sections through the series the impression is gained that the ectoplacental cone of one of the egg-cylinders overlaps that of the other in such a way that in the plane of the sections obtained, one seems continuous with the other, as represented in the figure. The boundary between the two is not distinct, and it would seem that as a result of pressure, partial fusion of the two had taken place. The presence of two egg-cylinders, enclosed within a single decidual crypt, as shown in this figure, with one of them having much smaller size and representing a younger stage of development, I believe is

not to be explained on the supposition of superfecundation or superfoetation. The record for this rat does not show insemination on successive days. At The Wistar Institute, after all of the supposedly successful matings of albino rats, the females rats are caged apart from the males. The smaller egg-cylinder, though appreciably smaller, is in stage of development separated from the other by a time interval of perhaps less than 24 hours. It presents a stage of development which is comparable to C of figure 27 (8 days) and except for size, to the one figured in figure 29 (8 days, 17 hours) of Part I. It is believed that in this case both ova were seminated at about the same time, and proceeded through normal segmentation and that on reaching the lumen of the uterus during the fifth day they became lodged in close proximity in the same mucosal fold. With the development of the decidual crypts, both became enclosed within the same crypt, at perhaps slightly different levels. In further development one blastodermic vesicle dominated the other and from about the seventh day on, one developed and differentiated more rapidly than the other. Had development continued, two distinct embryos, with separate amniotic cavities, attached to the same placenta, would have been formed, with one embryo large and more fully developed than the other. From mere difference in size and of development of embryos in the same litter it is not warranted to postulate superfecundation nor superfoetation. I am of the opinion that usually when two morula masses are lodged in close proximity in the same mucosal fold, one or the other degenerates (fig. 2, A) and that the normal development of both, as in the preparation shown in figure 10, is of very rare occurrence.

CONCLUSIONS

A study of the abnormal or pathologic ova met with in the extended series of preparations covering the first ten days of the development of the albino rat, enables grouping them in two main classes:

a. Such in which all of the ova of a given rat show, or are associated with, abnormal development.

b. Such in which a single abnormal or pathologic ovum is found in the same uterus along with an average number of normally developed ova.

When all the ova in a given uterus show abnormality, the presumption seems warranted that the underlying cause of the abnormality is to be sought in an altered or pathologic condition of the uterine mucosa. In the instances observed, the presence of maternal blood with many phagocytic leucocytes was noted in the lumen of the uterus, adhering to and surrounding the ova. From the study of sections of the uteri of an appreciable number of albino rats, in which insemination and supposedly semination seemed normal, but in which on complete serial sectioning of the uterine tubes no ova were found, but in the lumen of the uterine tubes of which the presence of maternal blood and phagocytic leucocytes was noted, the conclusion seems warranted that death and complete absorption of ova, after a given stage of normal development has been reached, may occur. In such cases, one may with propriety speak of faulty implantation, due to altered or pathologic condition of the uterine mucosa, even in cases where no actual implantation would have occurred in corresponding normal stages. In the two rats (Nos. 91 and 104) in which this condition was observed, the decidual crypts were shallow and not developed to the extent normal for the respective stages, evidencing the abnormal condition of the mucosa.

In cases in which a single abnormal or pathologic ovum is found in the uterus along with several normal ova, the presumption seems justified that the underlying cause responsible for the abnormal development is to be sought in the ovum itself, and not in its environs.

Abnormal developmental stages, interpreted as due to irregular or retarded segmentation, irregular or abnormal segmentation cavity formation, and retarded development of the ectodermal node and primary embryonic ectoderm, where only a single ovum shows abnormal development in a uterus containing the average number of ova presenting normal development, are difficult to explain on the assumption that extraneous influences affecting a single ovum are operative. Practically all

of the abnormal ova of the class described, and especially is this true for older stages, present normal relations to the uterine mucosa and the walls of the decidual crypt after implantation, and so far as may be determined by structure, give evidence of normal absorption of maternal hemoglobin in stages in which such absorption is pertinent. It may be argued that a single ovum may be less favorably placed in relation to embryotroph or pabulum, and as a result of unfavorable nutrition, develop abnormally. This is difficult to conceive for stages in which the ova lie free in the lumen of the uterus, namely, to about the beginning of the seventh day after the beginning of insemination, when embryotroph or pabulum must be relatively evenly distributed. The presumption, it would seem to me, in such cases is in favor of regarding the primary cause of the abnormal development as inherent in the ovum.

Separation of the first two blastomeres and the presence of two egg-cylinders in a single decidual crypt are regarded as chance findings and as of rare occurrence, since each was met with only once in the material at hand.

LITERATURE CITED

Literature on pathologic ova of the albino rat is lacking. For the literature of all but the more recent work, dealing with comparative experimental teratology, the bibliographies accompanying the chapters of O. and R. Hertwig may be consulted; for that dealing with the pathology of human ova, the bibliographies accompanying the contributions of F. P. Mall may be consulted.

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COMMENT ON MISS BECKWITH'S PAPER ON "THE GENESIS OF THE PLASMA-STRUCTURE IN HYDRACTINIA ECHINATA"

MARIANNA VAN HERWERDEN

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In a recent article¹ discussing the origin and relationships of the protoplasmic granules in the egg of *Hydractinia echinata*, Miss Cora J. Beckwith gives a totally erroneous idea of the results of a study on the eggs of *Strongylocentrotus lividus* and some other Echinoderms which I published in the *Archiv für Zellforschung*, Band 10, 1913.

It seems probable that she did not see my original article, otherwise her conclusion (p. 215) that I consider the basophilic granules in the cytoplasm as an artefact (because I could not have seen them in the living egg) would have been utterly impossible. In reality, I emphasize in my paper that I have seen these granules in young, living egg-cells (*Arch. Zellforsch.*, Bd. 10, p. 438) and that they later form the building-stones of the so-called chromidia of the German authors. All that I considered as an artefact was *the particular way in which the chromidia are arranged around the nucleus*, this appearing in a most pronounced manner after insufficient fixation methods. Had she read pages 438 and 439, she would have seen that I agree with her (p. 200) that with good fixation there is a uniform distribution of the granules.

On page 215 Miss Beckwith says: "Unlike Schaxel, however, he (van Herwerden) holds the mitochondria to be developed from this basophilic substance, since it is also a nucleinic acid compound;" and (p. 216) "the nuclease digestion indicates nucleinic acid present in the granules and mitochondria." Anyone will search my paper in vain for a chemical test for mitochondrial constitution. I have never attempted to give one. Having demonstrated the nucleinic-acid nature of the granules composing the particular structures called chromidia, I only said that, with the Benda stain for mitochondria, I obtained violet-colored granules on the alveolar walls which, located as they are, probably are to be considered as the same elements we recognized in the alcohol-fixed preparations as containing nucleinic acid. The doubts I expressed in my paper on the specificity of the Benda-stain reaction (see, also, *Ciona intestinalis*) was reason enough for avoiding the use of the term mitochondria instead of basophilic granules. I only stated that, using Benda, the deep stain of the granules situated

¹ *Jour. Morph.*, vol. 25, no. 2, 1914

on the alveolar walls, suggests that they may be identical with the building stones of the chromidia. But every reader of my paper will understand that this is not the essential point of my argument in discussing Schaxel's emission hypothesis.

I quite agree with Miss Beckwith that similarity in staining reactions is not sufficient for the identification of materials. Emphasizing this very point in my paper, I introduced the nuclease digestion as a convincing microchemical test, and I only regret that Miss Beckwith did not make use of this method, for I believe that for this special case it would have had a greater value than her attempts to accept or reject the identity of the chromatin and the basophilic granules in the cytoplasm.

To avoid any misconception, I must add, further, that the demonstration of nucleic-acid compounds in the cytoplasm of the egg of the sea-urchin does not in any way imply their introduction from the nucleus; it is possible that they have been generated *in loco* by chemical changes.

THE AUTHOR'S REPLY

In reply to Dr. van Herwerden's comments may I say that I regret exceedingly having, in my brief summary, in any degree misinterpreted her article. That the difficulty is due to a misunderstanding both on my part and hers, I shall hope to show. I have reread with great care Dr. van Herwerden's article (Arch. für Zellforsch., Bd. 10, 1913) to see wherein I have failed to understand it. In regard to the first point mentioned, i.e., that I quoted her as saying that the basophilic granules are artifacts, it is evident that my summary, as well as her original statement, is none too clear. I find no reference to an earlier article in which she believes that that point is evident. That I understood basophilic granules to be visible in the *living mature egg*, and so stated, is seen from a sentence preceding the one she quoted (p. 215) in which I said that she had described the mitochondria (basophilic granules) as visible in life in the mature egg. May I correct here her impression regarding a minor point. I did not say, as she quotes me as saying, that she "*could not have seen them in the living egg*," but that she *did not see them in the young living egg*. This last statement of mine, that she did not see the basophilic granules in the *young living oocyte*, she may question with justice. I evidently mistook her statement to the effect that *some* young cells showed no granules to mean *all* cells (p. 440) and my conclusion is therefore erroneous on that point.

In regard to my further statement that the chromidia of fixed material are artifacts, in view of Dr. van Herwerden's more recent comment, I see that I misinterpreted the following sentence (p. 439); "Ich halte also die Chromidienstruktur dieser Zellen für ein Kunstproduct. Die Herkunft und die Bedeutung der basophilen Körner in den Eizellen, welche die Baustein dieser Chromidien bilden etc.," having understood

'Chromidienstruktur' to mean chromidial structure rather than chromidial arrangement as was evidently intended.

Again, I have not intended to indicate and cannot see that I indicated that Dr. van Herwerden made a chemical test of mitochondria. As to her taking exception to my statement that she said that "nuclease digestion indicates nucleic acid present in the granules and mitochondria" I can only say that I based that conclusion on the sentences (p. 446): "Untersucht man das reife Ei von *Strongylocentrotus lividus* und *Holothuria tubulosa* nach der Bendaschen Methode, so trifft man violette Körner auf den Wabenwänden des Zellplasma zwischen den blassen Dotterkörnern gelagert an, welche höchst wahrscheinlich dieselben Elemente sind, die wir im Alkoholpräparat als nucleinsäurehaltende Körner kennen lernten, welche nach der Nucleasawirkung verschwanden. Ich meine also in Gegensatz zu Schaxel, dass in diesem Fall die als Mitochondrien im reifen Ei dieser Tiere beschriebenen Elemente mit den nucleinsäurehaltenden Bausteinen der sogenannten Chromidien identisch sind." If I have taken her 'höchst wahrscheinlich' to mean more than she intended, I can but apologise.

I can only be glad that the difficulty has been largely one of misinterpretation and that on the whole our results substantiate one another.

CORA JIPSON BECKWITH

THE DEVELOPMENT OF THE HYPOPHYSIS IN SQUALUS ACANTHIAS

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FORTY-THREE FIGURES

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INTRODUCTION

Although considerable work has been done on the hypophysis of elasmobranchs, many points still remain concerning which there has been much discussion and which evidently require further investigation. It was thought that an intensive study of the history of this organ in one form might be of value.

The embryos used are from the collection of Dr. R. E. Scammon and from the large series which form a part of the embryo-

¹ This work was begun while at the University of Minnesota and completed at Washington University Medical School.

logical collection of the Harvard Medical School.² For the pup and adult stages special sections and dissections were made. The difficulties in making a dissection of the hypophysis in selachians have been noted by other investigators. In the present work, it was possible only after numerous attempts to obtain a dissection showing the ventral lobes connected with the rest of the hypophysis. Graphic and wax reconstructions of different embryos and parts of the adult *Squalus* were made.

LITERATURE

1. *Embryology*

The literature on the development of the elosmobranch hypophysis may properly be divided into two categories, one on the embryology and the other on the adult anatomy and histology of this organ. Although the earlier work on the hypophysis in elasmobranchs concerned its adult anatomy, probably investigators have occupied themselves more with its development. Some, of course, have given attention to both the adult anatomy and the development in the same paper or in a series of articles.

Müller ('71) confirmed Rathke's earlier observations that the hypophysis is developed from the mouth. In *Acanthias vulgaris* and other selachians he found that it was composed of a principal posterior part and a secondary anterior part. He described the position and gave measurements of the size of the hypophysis and of the thickness of different parts of the wall in an *Acanthias* embryo 30 mm. long. In a 10 cm. embryo of *Mustelus* he described the tubular masses forming the hypophysis.

Balfour ('74) briefly stated that the hypophysis is an out-pouching from the mouth. In 1878 he described the hypophysis as being somewhat constricted from the mouth by the close of

² Through the courtesy of the late Dr. Charles S. Minot I was able to make use of the extensive Harvard Embryological Collection and had the privileges of his laboratory during the summer of 1914. I also wish to thank Dr. J. S. Kingsley and others of the South Harpswell Laboratory for the courtesies of that laboratory for a part of the summer.

'Stage K.' Soon after this its terminal part dilates and the hypophysis is completely constricted from the mouth.

Reichert ('77) in a description of some cleared embryos of *Acanthias* remarked on the position of the hypophysis and its relation to the notochord. He opposed the theory of the ectodermal origin of the hypophysis in *Acanthias*.

Rabl-Rückhard ('80) in his work on the relations of the notochord, confirmed Müller's observations concerning the hypophysis. He stated that there is a ventral secondary outpouching from the hypophysis in a 60 mm. *Acanthias*. In an embryo corresponding to Stage K of Balfour he has perhaps mistaken the connection between the premandibular somites for the hypophysis. This seems probable from the position as well as from the fact that he found no connection between this structure and the mouth at this time.

In 1888 Eddinger published a paper on the comparative anatomy of the forebrain. He stated that in *Torpedo* the hypophysis is at first a simple outpouching of the mouth which later develops secondary outpouchings.

Sedgwick ('92) in his work on *Scyllium* and *Raia* made the statement that the first rudiment of the mouth extends into the pituitary body.

Von Kupffer ('94) reviewed the literature on the development of the hypophysis and gave some results of his own work. He thought that the hypophysis is not derived entirely from ectoderm, and his observations on other vertebrates supported his view that the hypophysis is partly of entodermal origin.

Hoffmann ('96) described the early stages in *Acanthias* development. He stated that the hypophysis develops entirely from ectoderm. He found the first definite outpouching anterior to the buccopharyngeal membrane in 13 to 14 mm. embryos. He also described a yellowish pigment in the buccopharyngeal membrane which could still be seen in the stalk of 25 mm. embryos.

Ha'ller ('96) gave an account of the development of the hypophysis in *Mustelus*. He described the position of the organ in a 22 mm. embryo and called attention to the low epithelium found at the opening of the hypophysis into the mouth. The

connection with the mouth is soon lost and in a 90 mm. stage the whole structure has come into closer relation with the brain floor and vascular sac, and has grown forward. The roof of the hypophysis has become thickened, as have the anterior and posterior ends. From the floor of the caudal end two lateral outpouchings have developed which later form the inferior sacs. In a 20 cm. *Mustelus* all parts of the hypophysis are quite well developed. A vascular layer separates the roof from the brain floor. The roof of the inferior sacs becomes thinner. Haller also described the interhypophyseal canal joining the inferior sacs to the superior part. He figured glandular outgrowths extending forward from it. From the floor of the anterior sac are several prolongations extending anteriorly while from the roof are many small outpouchings. The extreme anterior end shows many mitotic figures and is broken up into a network by many capillaries. The floor at the cephalic end of the anterior lobe is very thin—this, Haller stated, is where the hypophysis is constricted from the mouth and here the epithelium has remained of a low cuboidal type. The head of the organ is composed of many closely-crowded tubules. These develop first as solid evaginations, then the nuclei separate and rearrange themselves, later a cleft appears in the protoplasm, which cleft ultimately connects with the main lumen.

Chiarugi ('98) briefly considered the hypophysis in a paper on the description of a prehypophyseal body and the hypophyseal area in *Torpedo ocellata*. He described a connection between the premandibular somites and the hypophysis and figured a median sagittal section of a 15 mm. embryo which showed a constriction of the hypophysis from the mouth.

Nishikawa ('99) noted that the hypophysis is present as a simple outpouching in 32 mm. *Chlamydoselachus* embryos. He made a series of drawings of transverse sections which show its position at this stage.

Sewertzoff ('99) in studying the development of the selachian skull took up the interrelation of development of the skull and brain. The trabeculae and parachordal plates in *Acanthias* and *Pristiurus* are almost at right angles to each other in early

stages (figs. 25 and 29). Later a bend in the medulla and a corresponding one in the parachordal plate changes the relation of the two cartilages. Also the forebrain has shifted from a position ventral to the medulla to a more dorsal and rostral one (fig. 1). Sewertzoff showed the change in position of the hypophysis occurring with the change in position of the skull and brain (fig. 23).

Rossi ('02) described the development of two lateral lobes in *Torpedo*. These, he stated, develop very early from the lateral walls of the evagination forming the hypophysis. He homologized the different portions of the hypophysis as he found them with those described by Haller, but described two special lateral lobes which he stated have no homologous parts in *Mustelus* according to Haller's description.

Gentes ('06, '07) found a close relationship between the vascular sac and the underlying hypophysis. In several short reports ('08) he described the lateral lobes and the development of the inferior lobes of the hypophysis. These two parts, he stated, form a ventral pituitary body. In a longer paper this author ('08) gave the results of his studies on the development and evolution of the hypophysis in *Torpedo*. He described two main parts, the superior and inferior sacs. The superior sac is further divided into posterior and anterior parts. The hypophysis begins as an outpouching which in 45 mm. embryos shows a beginning of its division into the two main sacs. From the posterior part of the superior sac many cords grow dorsalward, these later becoming tubular. Gentes homologized the superior lobe with the anterior lobe of mammals. The lateral lobes he believed persist through life.

Ziegler ('08) in a series of figures of an embryo of *Chlamydoselachus*, corresponding to Balfour's Stage L-M, showed the hypophysis as an upward anterior-extending evagination from the mouth. The connection with the mouth is still a wide canal.

Johnston ('09) described the hypophysis in *Acanthias* as consisting of a short anterior portion which grows toward the optic chiasma, and a longer posterior lobe directed toward the vascular sac.

Scammon ('11) in the normal plate series has figured the hypophysis in several of the younger stages. He described a distinct outpouching in 7.5 mm. embryos. In 18 mm. embryos a constriction of the anterior part of the hypophysis from the mouth has begun. In 24 mm. embryos shallow furrows separate two lateral portions from a median portion in the posterior part. A slight lateral constriction separates the anterior and posterior lobes.

In 1912 Sterzi described the development of the hypophysis in *Acanthias*. He noted that the front wall of the outpouching becomes dorsal in later embryos. A rostral lobe develops which is in part anterior to the stalk connecting the hypophysis to the mouth. Two lateral outpouchings arise which later form the endocranial portion. The dorsal lobe develops at the superior end of the early outpouching. An early differentiation takes place in the cells of the dorsal lobe. Buds grow out from this thickened wall and form epithelial cords between which are blood vessels and nerve fibers. Differences in the affinity for stains distinguish the rostral (and endocranial) lobes from the superior which is the chromophobic lobe in adults.

2. Anatomy and histology

Von Michlucho-Maclay ('68) described in *Acanthias* and in *Scymnus* a persistent connection between the mouth and the hypophysis. In his later work ('70) this investigator failed to find such a connection and believed his former observations to be incorrect.

Müller ('71) found in later embryos and adults of *Acanthias* that the cells forming the glandular part are columnar while those toward the periphery of the cords and tubules are spindle-shaped, with a finely granular cytoplasm. He also noted the large capillaries and the connective tissue between the tubules.

Viault ('76) briefly described the hypophysis in *Raia*. The hypophysis is surrounded by a connective tissue sheath which sends strands into the organ carrying large capillaries between the convoluted tubules. These tubules are 0.015 to 0.007 mm.

in diameter and usually have a small lumen. They are composed of cylindrical cells. The tongue-like anterior end, he stated, was of similar structure. He was of the opinion that the glandular portion arose from the mouth.

Rohon ('79) described an hypophysis composed of tubules in selachians. It is a triangular shaped organ with a long tongue-like projection which extends forward almost to the optic chiasma. He found no cavity within it. In *Acanthias* and *Mustelus* the hypophysis is larger than in *Torpedo* and *Scyllium*.

Sanders ('86) studied the central nervous system of *Scyllium* and *Acanthias*. He stated that the hypophysis is attached to the infundibulum by a glandular tube which lies between the inferior lobes. In a drawing of the brain of *Acanthias* (lateral view) he figured the hypophysis as lying caudal to the inferior lobes of the mesencephalon.

In 1892 Edinge described the mid-brain region. In *Torpedo* and *Scyllium* he found the hypophysis composed of tubules and cords of epithelium, among which are many blood vessels. He noted nerve fibers extending from the infundibular region ventralward into the hypophysis.

Haller ('96) described in detail the structure of the hypophysis of a 20 cm. *Mustelus*. He stated that in the adult the glands of the head (superior) portion are tubular. Secretion and cell detritus are found in the lumina. He also described an opening in the base of the anterior lobe. This is found in the thin portion of the floor in the position of the original connection with the mouth, and connects the cavity of the hypophysis with the subdural space.

Sterzi ('04) described the hypophysis in several selachians. He found the anterior part flattened dorso-ventrally, extending almost to the optic chiasma and containing a large cavity. The posterior part is separated from the remainder by connective tissue. A slender canal connects this to the anterior portion. The superior part is formed of anastomosing cords interlacing with sinusoids. The cords are formed of a peripheral layer of columnar cells and an inner mass of polyhedral ones. The nuclei of the outer zone are rich in chromatin. The

polyhedral cells contain small spherical nuclei. The cytoplasm is granular and stains deeply.

Pettit ('06) made a study of the hypophysis in *Centroscymnus*. He mentioned posterior, median and anterior parts, all communicating. He found ramifying cords or tubules surrounded by sinusoids.

Burekhardt ('07, '11) in his work on the central nervous system of selachians described, incidentally, the hypophysis in *Scymnus*. He recognized a terminal, a median and a posterior lobe. Apparently he included under his division of median lobe, the caudal end of the anterior lobe of Sterzi, or it may be that the hypophysis in *Scymnus* is quite different from that found in other forms.

Joris ('08, '09) described the dorsal lobe in *Spinax* and *Mustelus* as formed in part from the hypophyseal evagination and in part from the infundibulum. The cell cords arise from the hypophysis while neuroglia and nerve fibres from the infundibular region grow in between these.

In 1909 Sterzi in his comprehensive work on the central nervous system of selachians, gave a clear description of the hypophysis. According to this description the hypophysis is composed of perimeningeal and endocranial parts. The perimeningeal portion is further subdivided into a dorsal and a rostral lobe. The dorsal lobe has many columns from its dorsal surface. Among these are numerous capillaries. The rostral lobe is a long flattened part extending almost to the optic chiasma. The endocranial part is composed of two sacs connected medially to one canal which leads to the anterior or rostral lobe. The cords and tubules of the dorsal lobe anastomose forming a network in which is a rich vascular supply. These are primarily attached by means of connective tissue to the base of the brain or vascular sac. The structure of these cords, Sterzi had described before ('04). The dorsal lobe, because it stained so lightly he termed the chromophobic portion. The rostral lobe has tubules extending from its ventral walls. The ventral wall has a distinct median ventral furrow and the tubules of either side

remain separated. The distal ends of the tubules are solid and by means of branches anastomose with the cords of the other tubules. The arrangement of the cells here is the same as that in the dorsal lobe. This portion stains more deeply, the capillaries are smaller and less numerous. The endocranial portion in embryos is a sac with folded walls. In the adult, many tubules and cords have developed. In the main this resembles the rostral portion of the perimeningeal part.

Tilney ('11) in his studies on the comparative histology of the hypophysis, stated that in *Acanthias* there is a distal epithelial portion made up of parallel cell columns. The cells are deeply acidophilic with only a few faintly-staining acidophilic ones present. In the juxta-neural part the cells are larger, usually irregularly disposed, but forming some acini. However, they take the basic stains very markedly. He found this portion less vascular than the distal part. Tilney has homologized the different portions of the hypophysis in the various vertebrate groups. The distal epithelial portion of selachians corresponds to the intermediate lobe of mammals.

In 1913 Stendell made a comparative study of the hypophysis. He described intermediate and main lobes in *Heptanchus*. In the main lobe he described the peripheral cells of the tubules as of an acidophilic nature, while those toward the center are basophilic or neutral. Stendell has homologized the parts found in the hypophysis of selachians with those in the higher forms of vertebrates. His homology agrees with that of Tilney, i.e., the intermediate lobe of *Heptanchus* is homologous with the same portion in the higher vertebrates, being in *Heptanchus* very large and becoming smaller in the higher forms. The ventral sac of the main lobe is not found in higher forms.

There have been many terms used in the descriptions of the different parts of the elasmobranch hypophysis. A comparison with figure 1, which is a drawing of a model of the hypophysis of a pup, and a table of the terms employed may serve to explain the terms used in reviewing the literature.

TABLE 1

Terms used by the various investigators for the different parts of the elasmobranch hypophysis

OBSERVER	MATERIAL	REGIONS OF THE HYPOPHYSIS			
		Anterior	Inferior	Superior	Special portions
Müller ('71).....	Scymnus, Acanthias	Anterior part (secondary)	Posterior	(main part)	
Haller ('96).....	Mustelus	Superior sac	Inferior sac	Head	
Rossi ('02).....	Torpedo	Median lobe; antero-median diverticulum	Antero-lateral diverticulum	Terminal lobe	Lateral lobes
Sterzi ('04).....	Mustelus, Acanthias	Anterior portion	Posterior portion	Superior portion	
Pettit ('06).....	Centro-scyrnus	Anterior	Median	Posterior	
Burckhardt ('07)...	Scymnus	Terminal lobe	Posterior lobe	Median lobe	
Gentes ('07).....	Torpedo	Superior sac; anterior two-thirds	Inferior sac	Superior sac; posterior one-third	
Joris ('08).....	Mustelus		Posterior	Dorsal	
Sterzi ('09).....	Acanthias	Perimeningeal portion; rostral lobe	Endocranial portion	Perimeningeal portion; dorsal lobe	
Tilney ('11).....	Acanthias	Juxta-neural part		Distal part	
Stendell ('13).....	Heptanchus	Main lobe	Ventral sac of main lobe	Intermediate lobe	
Sterzi, Gentes, et al.....		Chromophilic portion		Chromophobic portion	

MORPHOLOGY AND MORPHOGENESIS

1. Description of the hypophysis in the pup stage

A description of the hypophysis in the pup stage may make clear the different parts of this organ. The formation of tubules has begun and the main portions or lobes are well formed at this time.

The anterior lobe is a tongue-like process with two somewhat wider extremities; these are connected by a more slender middle part which is less than one-third of the entire length. There is a deep sulcus in the median ventral wall which extends from about the middle of the posterior extremity to near the end of the anterior extremity (fig. 1). Several other more or less regular furrows occur in the ventral surface of the anterior extremity. Two lateral constrictions separate the middle portion from the some-

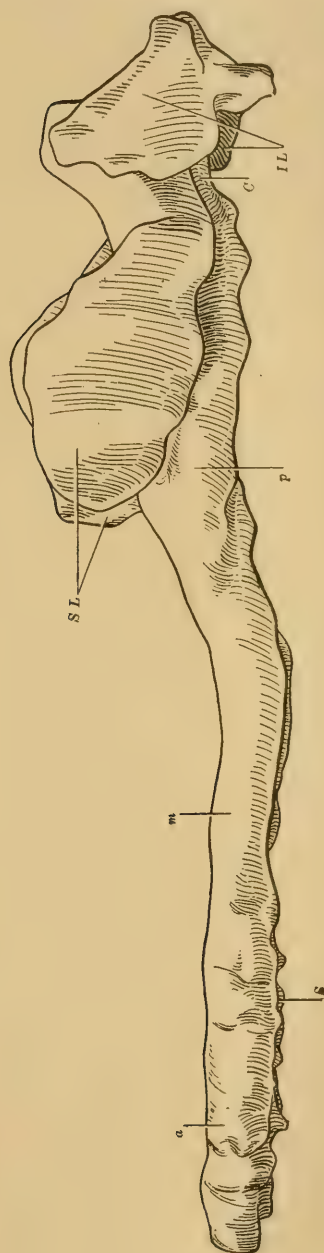


Fig. 1 Left lateral view of a reconstruction of the hypophysis of an *Acanthias* pup. $\times 50$. *a*, *p*, anterior and posterior extremities of the anterior lobe; *C*, interhypophyseal canal; *IL*, inferior lobe; *m*, mid part of anterior lobe; *S*, median ventral sulcus; *SL*, superior lobe.

what more dorsal posterior extremity. These extend as furrows posteriorly from the lateral dorsal side of the caudal end of the middle part (fig. 25). As just stated, the posterior extremity is somewhat dorsal to the other parts of the anterior lobe and is directly ventral to the superior lobe. Two deep horizontal furrows constrict the connection between the posterior extremity and the superior lobe.

The superior lobe is just below the saccus vasculosus to which it is closely attached, and is almost three times as wide as the posterior extremity of the anterior lobe (fig. 25). Its median part is about as long as the posterior extremity of the anterior lobe, but projects beyond it caudally and laterally. The caudal end extends somewhat dorsally. The lateral parts, or wings, of the superior lobe project somewhat upward and forward.

The inferior sacs extend laterally from a constricted median connection. They are much smaller than the wings of the superior lobe, but, viewed from above, give the appearance of two lesser caudal wings. From their median connection a short slender canal extends in a slightly oblique direction upward and forward to connect with the anterior lobe (fig. 25).

Fig. 2 Sagittal section of the hypophysis of an 8 mm. embryo. $\times 40$ (H. E.C. 210). *H*, hypophysis; *I*, infundibulum; *Mo*, epithelium of mouth; *Mm*, median mass connecting the premandibular somites; *N*, notochord; *Po*, post optic groove.

Fig. 3 Sagittal section of the hypophysis of a 15 mm. embryo. $\times 40$ (H.E.C. 228). For abbreviations, see figure 2.

Fig. 4 Sagittal section of the hypophysis of a 19 mm. embryo. $\times 40$ (H.E.C. 138). For abbreviations, see figure 2.

Fig. 5 Sagittal section of the hypophysis of a 22 mm. embryo. $\times 40$ (H.E.C. 231). *AL*, anterior lobe; *Med*, median connection of inferior lobes; *SL*, superior lobe; other abbreviations as in figure 2.

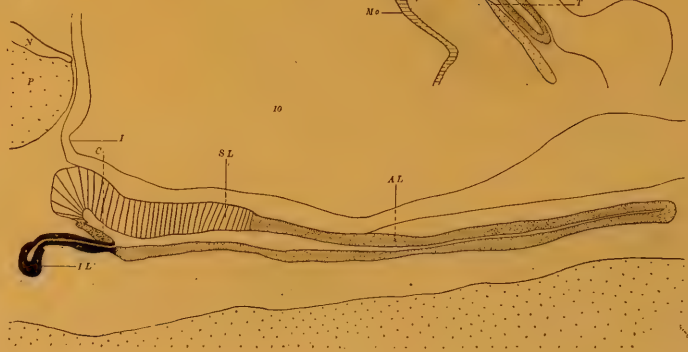
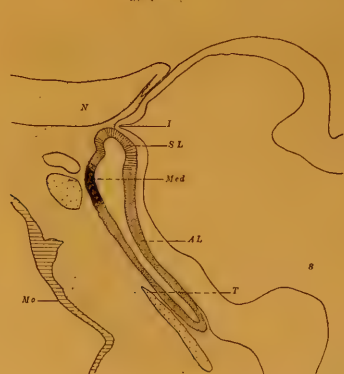
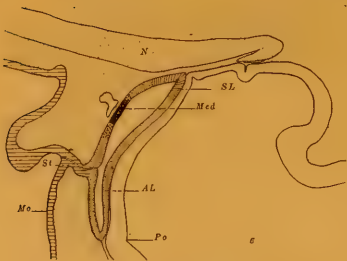
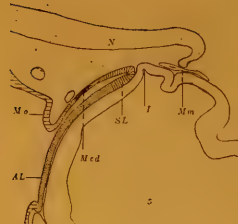
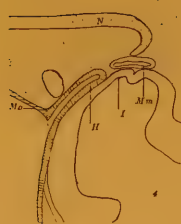
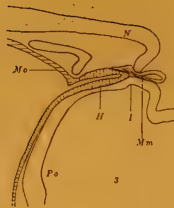
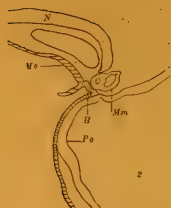
Fig. 6 Sagittal section of the hypophysis of a 28 mm. embryo. $\times 40$ (H.E.C. 234). *St*, hypophyseal stalk; other abbreviations as in figure 5.

Fig. 7 Sagittal section of the hypophysis of a 34 mm. embryo. $\times 40$ (H.E.C. 362). For abbreviations see figure 6.

Fig. 8 Sagittal section of the hypophysis of a 40 mm. embryo. $\times 40$ (H.E.C. 370). *P*, parachordal plate; *T*, trabeculae; other abbreviations as in figure 5.

Fig. 9 Sagittal section of the hypophysis of a 50 mm. embryo. $\times 40$ (H.E.C. 444). *C*, interhypophyseal canal; for other abbreviations, see figure 8.

Fig. 10 Sagittal section of the hypophysis of a pup, (reconstructed from transverse sections). $\times 40$. For abbreviations, see figure 8.



The position of the adult hypophysis has been described by Sterzi ('09) and others. The long tongue-like anterior lobe lies on the median ventral wall of the inferior lobes of the brain. Its anterior end extends forward almost to the optic chiasma. The superior part is placed posterior to this and in a more dorsal plane. It extends as far caudally as the saccus vasculosus. The inferior sacs of *Squalus* do not extend as far ventrally as has been described for other selachians. But they are ventral to the superior lobe and extend farther caudalward (fig. 10). From their middle connection a slender canal joins them to the ventral side of the caudal end of the anterior lobe.

2. Early development of the hypophysis

Recent work on the development of the hypophysis in elasmobranchs shows that it arises at an earlier period than was formerly believed. Hoffmann ('96) stated that the position of the future hypophysis is well marked in *Acanthias* embryos of 15 somites but that there is no indication of an evagination even in 8 mm. (50 somites) and 10 mm. embryos. Haller ('96) began his description of the hypophysis in *Mustelus* in embryos 22 mm. long. At that time, the hypophysis is already a distinct outpouching. More recently Johnston ('09) briefly described the earliest formation of the hypophysis in *Acanthias*. In an embryo of 24 somites the ectoderm from which the hypophysis develops is readily recognized. He stated that a short anterior lobe, developing later, extends toward the optic chiasma. Also, that the posterior part crowds between the brain and the median mass connecting the premandibular somites. Scammon ('11) mentioned a thickened hypophyseal plate in a 5.2 mm. embryo and a beginning evagination in a 6.2 mm. embryo (50-51 somites).

A median sagittal section of an *Acanthias* embryo 8 mm. in length³ is shown in figure 2. The anterior superior end is partially insinuated between the brain and the median mass connecting the premandibular somites, as had been noted by Johnston ('09) in about the same stage. During the time that the

³ In the description of the figures 'H. E. C.' has reference to the embryos of the Harvard Embryological Collection used for this study.

anterior part of the hypophyseal evagination is growing between the brain and the median mass connecting the premandibular somites, the posterior portion forms a more or less prominent ridge caudal to the connecting mass of the somites. A model of such an embryo shows as an evagination, the end of which is grooved transversely by the median mass connecting the premandibular somites (fig. 2). This groove may be quite prominent even in 11 to 12 mm. embryos and evidences of it are usually to be found at that time.

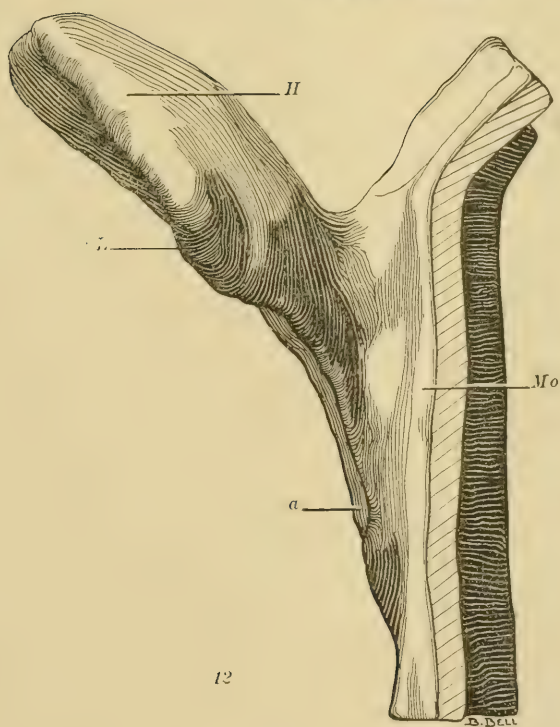
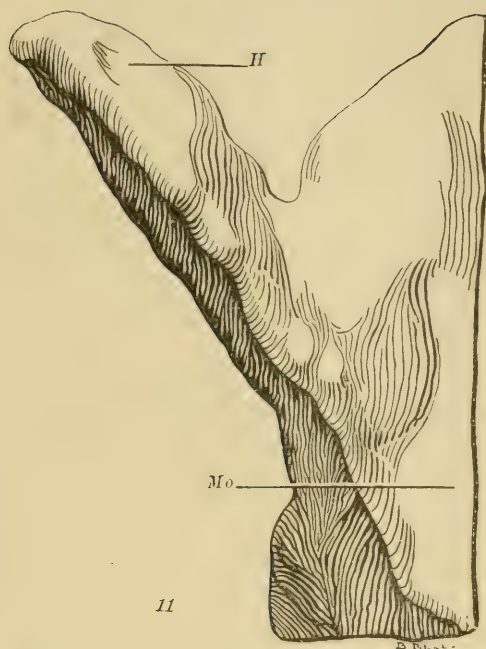
In 8 mm. embryos as well as in younger ones the anterior arm of the hypophyseal outpouching shows differentiation as far forward as the postoptic recess (fig. 2). Scammon ('11) stated that the notochord comes into contact with the early hypophyseal outpouching, and Sterzi ('12) has shown such a contact in *Mustelus* of 8 mm. (fig. 449). No such contact has been observed in this study of *Acanthias*.

A mid-sagittal section of a 15 mm. embryo shows that the superior end of the hypophysis has now grown well between the brain and the median mass connecting the premandibular somites. The evagination is more marked and the anterior arm is longer than in younger stages (fig. 3).

A model of the hypophysis of an *Acanthias* embryo 19 mm. in length is shown in figure 11; this view is taken from the left lateral side. The hypophysis at this stage is an anteriorly and dorsally directed outpouching, concave on its ventral surface where it lies in close relation to the diencephalon (fig. 18). Its superior anterior end extends to the infundibular recess. The opening from the pharynx into the hypophysis is small. A sagittal section at about the median line (fig. 4) shows that the thickened anterior (ventral) wall of the hypophysis extends forward as far as the postoptic groove as Johnston ('09) has figured it.

Fig. 11 Left lateral view of a reconstruction of the hypophysis of a 19 mm. embryo. $\times 130$. *Mo*, lining of mouth; *H*, hypophysis.

Fig. 12 Left lateral view of a reconstruction of the hypophysis of a 21 mm. embryo. $\times 100$. *a*, anlage of anterior end of hypophysis; *H*, hypophysis; *IL*, anlage of the inferior lobes; *Mo*, lining of mouth.



Several changes have taken place in the hypophysis of a 21 mm. embryo. It is still concave, both laterally and dorso-ventrally in its ventro-anterior surface (fig. 12). The thickened anterior wall of the hypophysis, reaching almost to the preoptic groove, is now distinctly evaginated. Scammon ('11) mentioned this closing off of the anterior part in a 20.6 mm. embryo and Sterzi ('12) described the formation of this 'rostral diverticulum' in 20 to 24 mm. embryos. The lateral side of the anterior outpouching is sharply demarcated by the formation of the stalk connecting the hypophysis to the mouth (fig. 19). The anterior end at this stage is almost half as wide as the posterior, from which most of the hypophysis is developed. The opening from the mouth into the early anlage is located as before, but is smaller now. A view of a model from the oral side shows that the constriction of the front and lateral sides of the anterior end of the anterior lobe has begun.

In a 22 mm. embryo the hypophysis (fig. 13) is not as concave as in younger stages. The anterior part shows laterally more marked constriction from the stalk which connects it to the buccal cavity. The anterior end is also markedly constricted. The opening into the hypophysis extends now from this anterior constriction to the posterior (caudal) margin of the opening into the first outpouching. The opening into the first evagination is very small and connects the pouch with the stalk (fig. 5). The posterior end is wider transversely than before. On its dorso-lateral surfaces are small ridges (fig. 13), the anlagen of the inferior sacs. On the ventral surface of the posterior part are two slight lateral furrows which are beginning to separate the inferior sacs from Rathke's pouch (fig. 20). These furrows are present in a 20.6 mm. embryo, as Scammon ('11) stated. The lateral pouches or inferior sacs appear as dilated cavities at either side. The grooves or furrows are as yet shallow and indistinct. In an 18 mm. embryo, Scammon ('11) noted the beginning of the division, by slight furrows, of the posterior portion into a median and two lateral parts. Some embryos do indicate the beginning division of the inferior sacs about that time but the furrows are not as prominent as the dilated cavities.

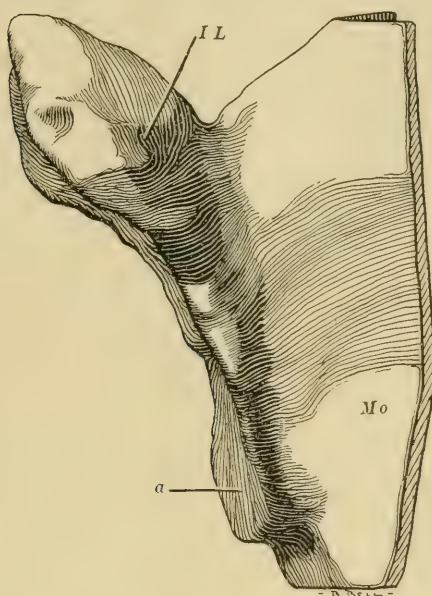


Fig. 13 Left lateral view of a reconstruction of the hypophysis of a 22 mm. embryo. $\times 100$. For abbreviations, see figure 12.

In a model of a 28 mm. embryo (fig. 14) the entire anterior portion is well closed off. It extends anteriorly almost to the postoptic recess. From its caudal surface a slender stalk connects the hypophysis with the mouth (fig. 6). The ventral end of the anterior lobe is somewhat wider than the mid part which connects it with the very much wider posterior portion or Rathke's pouch (fig. 21). Distinct furrows on the anterior (ventral) side partially separate the lateral anlagen of the inferior sacs from the original Rathke's pouch. A slight ridge on the caudal (superior) surface joins the two inferior sacs (fig. 14). In a median sagittal section (fig. 6) a shallow groove marks the connection between them. This connection is dorsal (caudal) to the stalk joining the hypophysis to the mouth. On the lateral dorsal surface at the posterior end of Rathke's pouch two very slight outpouchings indicate the position of the developing superior lobe (fig. 14).

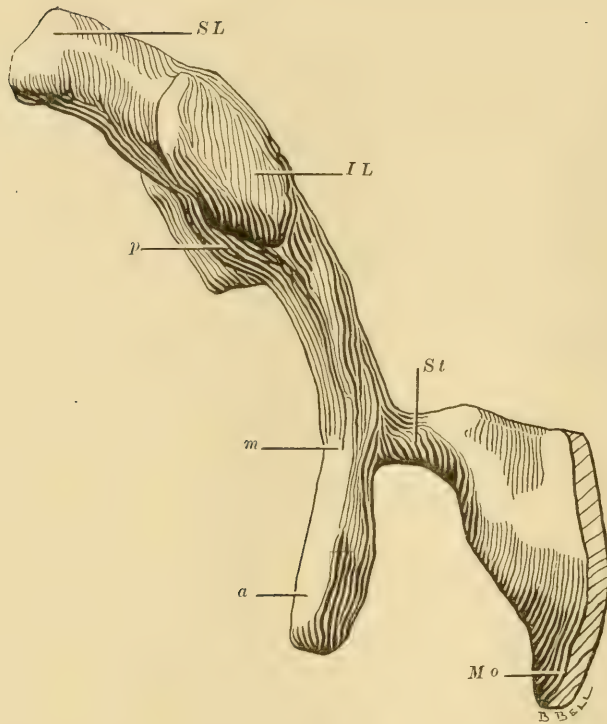


Fig. 14 Left lateral view of a reconstruction of the hypophysis of a 28 mm. embryo. $\times 100$. *a*, *p*, anterior and posterior extremities of anterior lobe; *IL*, inferior lobe; *m*, middle part of anterior lobe; *Mo*, wall of mouth; *St*, hypophyseal stalk; *SL*, anlage of superior lobe.

3. Later development of the hypophysis

All the main outpouchings of the hypophysis are present in the 28 mm. embryo. The inferior lobes are quite prominently marked off, the anterior lobe shows two widened extremities and the superior lobe has just begun to evaginate. The anterior end of the hypophysis in a 33 mm. embryo is curved slightly forward. The anterior lobe is not so markedly concave antero-posteriorly as in earlier stages (fig. 7). The ventral (anterior) furrows separating the inferior lobes from the posterior medial portion, are now quite deep (fig. 15). On the caudal side a distinct ridge extending between the inferior lobes indicates their

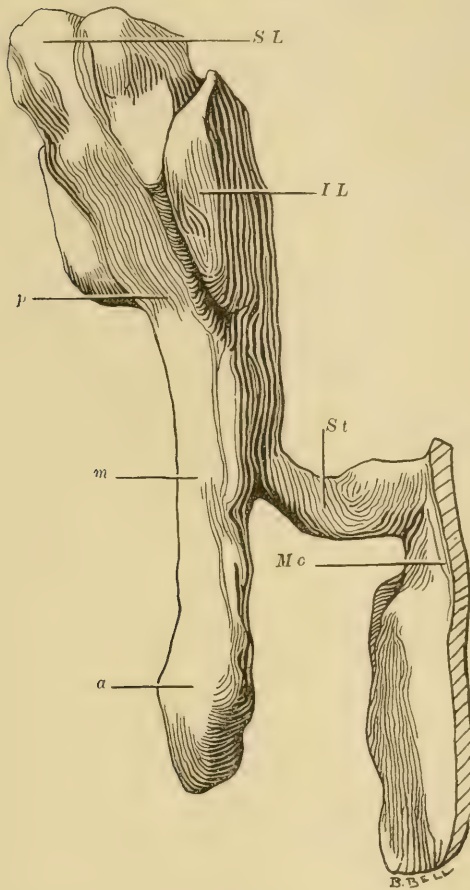


Fig. 15 Left lateral view of reconstruction of the hypophysis of a 33 mm. embryo. $\times 100$. For abbreviations, see figure 14.

early connection with each other. On the posterior tip of the hypophysis two lateral grooves mark the beginning constriction of the superior lobe (fig. 22), the outpouching of which was seen in the previous stage. The stalk connecting the hypophysis to the pharynx is smaller than in earlier stages.

The anterior lobe of the hypophysis, particularly its anterior part, has increased in length in a 40 mm. embryo. The stalk joining the hypophysis with the mouth has disappeared, with

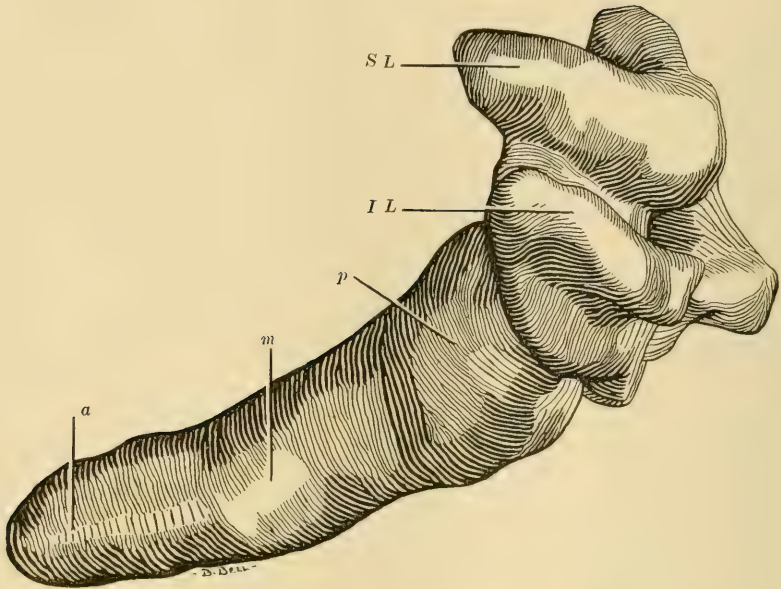


Fig. 16 Left lateral view of a reconstruction of the hypophysis of a 48 mm. embryo. $\times 100$. For abbreviations, see figure 14.

the exception of a cone-shaped mass of cells connected with the oral epithelium, and an irregular area in the floor of the hypophysis which represents the remains of the former attachment (fig. 8). The furrow uniting the two inferior lobes across the middle line is more marked, and the superior lobe also is prominent. The anterior end of the hypophysis now extends forward and downward. The straightening out of the head bend in the development of the embryo has probably helped to bring about this change.

More marked changes have taken place in a 48 mm. embryo (fig. 16). The anterior end of the anterior lobe is wider than in earlier stages. A short and narrow middle part connects this portion with the caudal extremity which is considerably wider (fig. 23). The inferior lobes are attached to the, now, ventral (caudal) side of this part. The ridge connecting the two inferior lobes has become very pronounced but still opens widely into

the anterior lobe (fig. 16). The furrows separating the inferior lobes from the anterior are much deeper and wider. The inferior lobes have enlarged in their dorso-ventral and in their transverse diameters, and they extend laterally beyond the posterior extremity of the anterior lobe. Marked development has taken place in the superior lobe. The lateral furrows separating it from the anterior one are deeper, and the cranio-caudal length of the lobe has increased so that there is a projection caudally beyond the anterior lobe. The antero-lateral ends of the superior lobe have grown forward.

The median connection of the inferior lobes is constricted from the posterior (ventral) part of the anterior lobe in a 50 mm. embryo. The inferior lobes are directly ventral to the superior lobe. There remains a short slender tube in the mid-line connecting the inferior lobes to the anterior (fig. 9). The duct connecting them to the anterior lobe extends almost straight anteriorly.

A median sagittal section of an 86 mm. embryo (fig. 27, G) shows an increase in the length of the hypophysis. The inferior lobes lie more caudally and the duct joining them to the anterior lobe is longer.

In a 95 mm. embryo the anterior lobe has increased greatly in length (fig. 17). A median ventral sulcus has appeared and the anterior third of this lobe is quite wide. A middle narrow portion, almost circular in cross section, connects the anterior extremity to a wider posterior end (fig. 24). The caudal extremity is connected dorsally with the superior lobe. The inferior lobes are continuous across the median line. The connection between the inferior lobes and the anterior one is a small tube which extends almost straight forward to join the inferior surface of the caudal end of the anterior lobe just below where this opens into the superior one (fig. 10). The inferior lobes have enlarged in their cranio-caudal axis. The lateral parts of the superior lobe have increased in their cranio-caudal diameters and extend forward beyond the median part. The latter has grown caudalward and lies just dorsal to the tube joining the inferior and anterior lobes.

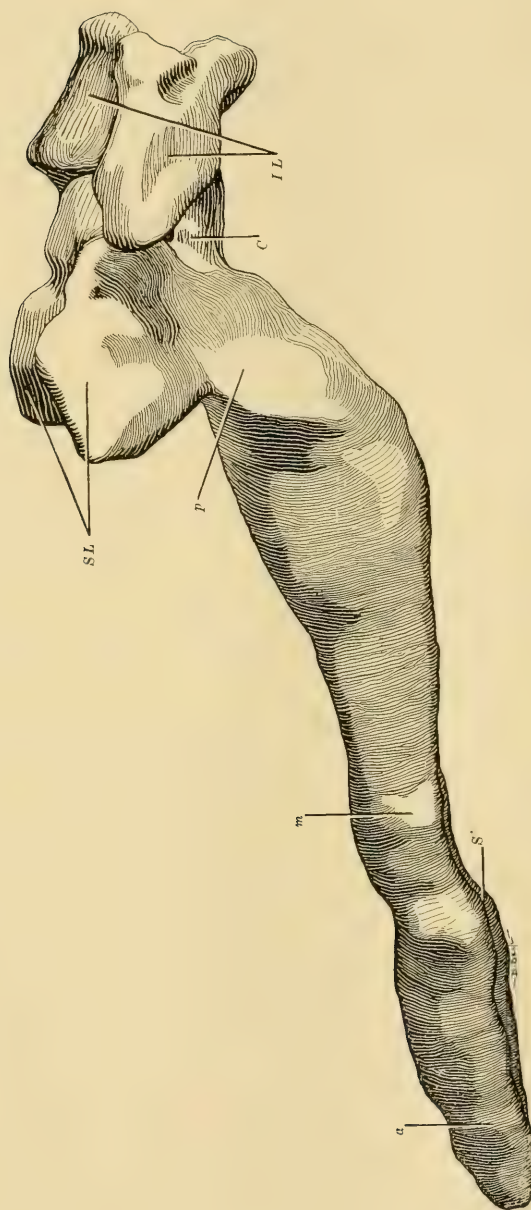
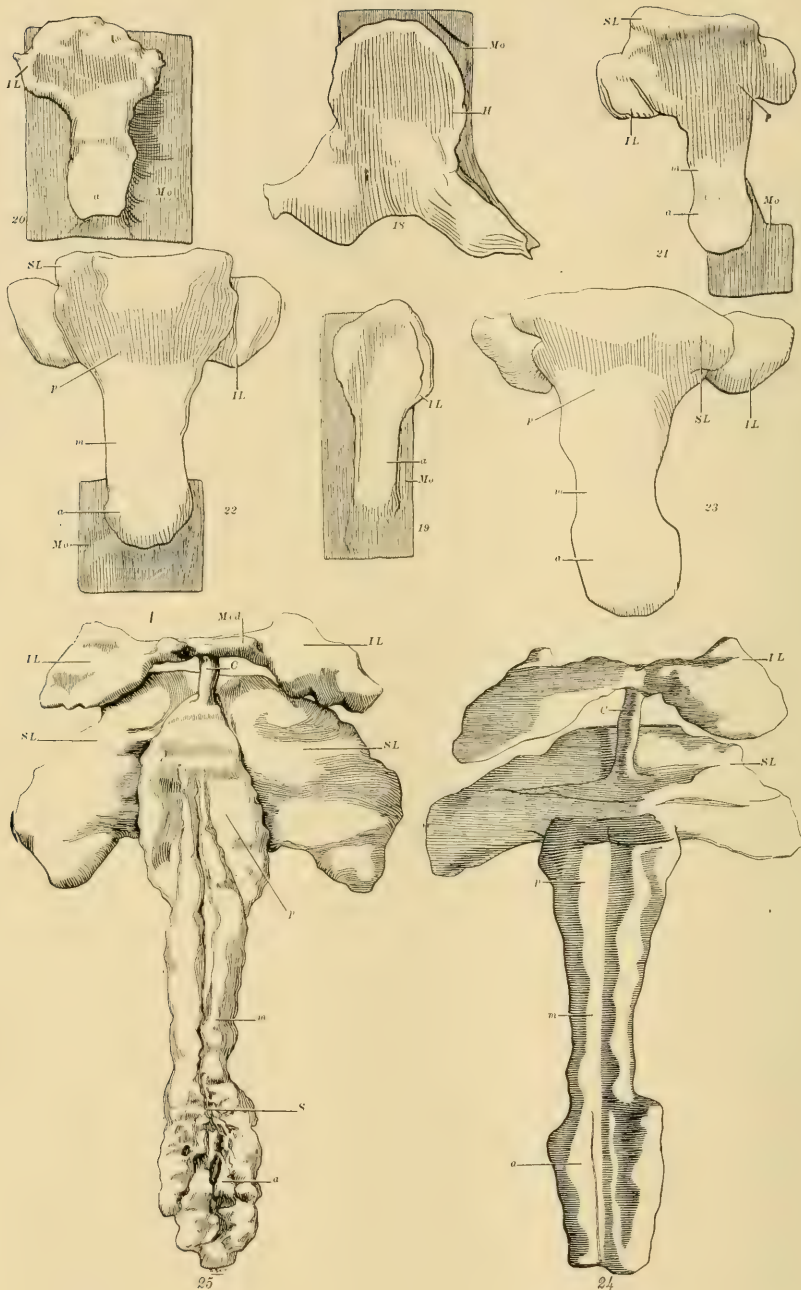


Fig. 17 Left lateral view of a reconstruction of the hypophysis of a 95 mm. embryo. $\times 75$. *C*, interhypophyseal canal; *S*, median ventral sulcus; other abbreviations as in figure 14.

4. The hypophysis of the adult

A detailed description of the pup stage has been given (p. 400) as typical of the morphology of the adult condition (fig. 1). A drawing of a dissection of the hypophysis of an adult will show the position and relation of some of the parts more clearly (fig. 26). The anterior end extends almost to the optic chiasma, as noted by Sterzi and others. From a study of the models of the 'pup' stage and of sections of adults it appears that the middle part of the anterior lobe is more than a mere constriction separating a larger rostral from a smaller caudal part as Sterzi ('09) described. In the pup this middle part is only a little shorter than either extremity, while in the adults it is much shorter. It is, however, distinctly marked. In some cases there are cystic outgrowths from the floor of this part; in others the walls and floor are quite regular and therefore are distinctly different from either extremity. The middle part is smaller than either extremity in its dorso-ventral and lateral diameters in pups (figs. 24, 25) and in adults, but the changes in diameter from either end to the middle part in adults takes place gradually and the parts are not so definitely marked, except in those cases in which no glandular outgrowths occur from the middle region which is much more prominent in sections and in wax reconstructions than is shown in dissections. The superior lobe projects some distance laterally on the ventral side of the vascular sac. It is difficult to make out the lateral limits of this part in the dissected specimen, as it seems to be continuous with the ventral surface of the vascular sac (fig. 26). In transverse sections, however, the lateral extent of the wings of the superior lobe is clear. The superior lobe is convex dorsally and closely attached to the saccus vasculosus. The latter dips down on either side of the caudal end of the anterior lobe and so partially separates the lateral wings of the superior from the anterior lobe.

The inferior lobes no longer resemble the sac-like structures with cystic outpouchings of the pup stage. Both superior and inferior walls have developed a mass of tubular-like glands. None of these was observed extending cranialward, as described by



Figs. 18-20 Antero-ventral views of the reconstructions shown in figures 11 to 13. Figure 18, $\times 50$; figures 19, 20, $\times 40$.

Figs. 21-23 Anterior views of the reconstruction shown in figures 14 to 16. $\times 40$.

Figs. 24-25 Ventral views of the reconstructions shown in figures 17 and 1. Figure 24, $\times 40$; figure 25, $\times 25$. For abbreviations, see figure 1.



Fig. 26 Drawing of a dissection of the hypophysis of an adult *Acanthias* from ventral side. $\times 20$. *AL*, anterior lobe; *B*, inferior lobes of the brain; *C*, interhypophyseal canal; *IL*, inferior lobes of the hypophysis; *SL*, superior lobe of the hypophysis; *VS*, vascular sac.

Haller in *Mustelus*. From their median connection a long slender tube extends to the caudal end of the floor of the anterior lobe. As seen in figure 26, the median connecting portion of the inferior lobes may be large. In some specimens this part, as well as the lobes, shows a mass of tubules. The caudal ends of the inferior lobes are surrounded by cartilage.

The shifting of the hypophysis with reference to its position in the body and the development of different parts is brought out very clearly in figure 27. Sewertzoff ('99), in his studies

on the development of the skull, made use of such a figure to show the interrelation of development of the brain and skull. He made an outline drawing of the skull and brain of an embryo, choosing an arbitrary magnification. He then made drawings of different sized embryos of such a magnification that points, corresponding to two arbitrarily chosen in the first drawing, would coincide. In figure 27 of this paper, the same scheme was adopted. The magnification of the first drawing was such as to avoid as much as possible the confusion of lines. The points chosen were the extreme anterior end of the notochord and the axis of the notochord at the level of the first spino-occipital nerve. All the other drawings were then made so that these two points—the extreme anterior end of the notochord and the axis of the notochord at the level of the first spino-occipital nerve—should coincide with those of the first drawing. The outlines of the hypophysis were then drawn, using a line between the two points as a base.

The objections to such a drawing are readily apparent. There are, of course, individual variations in the embryos. Also, these points are probably continually changing during development. For a comparative study, however, the variations can be no great objection and the points chosen are probably as reliable as any. A series of embryos from 11.5 mm. in length to the pup were drawn in this manner (fig. 27). One can see at a glance in all these stages the relative position of the hypophysis with reference to the anterior end of the notochord. Also, as was pointed out in the description of the different embryos, what is first the dorsal wall becomes in later stages the ventral, while the ventral or anterior becomes dorsal. The early superior end of the evagination shifts more and more caudalward with reference to the rest of the hypophysis, until, in the pup, the superior lobe, which develops from the superior dorsal end, is caudal in position. The inferior lobes, which develop from the sides of the superior end and are on the same horizontal plane as the superior position, take a position ventral to the superior lobe in the late embryo and adult. The furrows separating these inferior lobes, described as appearing in the 20 to 22 mm. embryos



Fig. 27 Composite drawing of median sagittal sections of the hypophysis of *Acanthias* embryos. *A*, 11.5 mm. embryo; *B*, 18 mm.; *C*, 34 mm.; *D*, 37 mm.; *E*, 40 mm.; *F*, 50 mm.; *G*, 86 mm.; *H*, pup. X-Y, base line drawn from the anterior end of the notochord to the axis of the notochord at the level of the first spinous occipital nerve.

on the ventral (anterior) side of the hypophysis, are later (50 mm. embryos) on the dorsal side. The anterior lobe, first directed almost vertically, grows ventrally and later extends more and more anteriorly until it is directed horizontally (cranio-caudally).

The comparative growth of the different portions is also made clear. The anterior lobe comprises all of the original outpouching and also the anterior tongue which evaginates later. The increase in length of the anterior lobe, particularly its anterior extremity, is marked. The inferior lobes, developing from the sides of the posterior portion of the early evagination, become continuous across the posterior side (34 mm. embryos) and finally constrict entirely except for a short duct connected with the anterior lobe. The inferior lobes increase greatly in size, but, in the adult, are largest transversely. The superior lobe, developed from the superior dorsal part, spreads far out transversely and later enlarges in its dorso-ventral axis.

The posterior extremity of the anterior lobe is then developed from the first outpouching. A little later (21 mm.) the part anterior or ventral to the original outgrowth evaginates, forming largely the middle narrower portion of the anterior lobe. The anterior extremity of the anterior lobe develops at the extreme anterior (ventral) end of this. The stalk connecting the hypophysis with the mouth is attached to the middle narrower portion. The connection between the inferior lobes develops caudal (dorsal) to this but arises from a part which later becomes the floor of the posterior extremity of the anterior lobe. From this description it is seen that the inferior lobes, developing from the posterior end of the hypophyseal anlage, and the superior lobe, from its extreme dorsal (anterior) end, are derived from a part of the anterior lobe. Such an explanation of the development of the parts is well borne out by a study of the models as well as of the various sections of embryos.

In comparing figures 6, 7 and 8 it is seen that the hypophyseal stalk in a 40 mm. embryo is attached nearer the caudal end of the hypophysis than it is in younger ones. This does not agree with what Haller has described for *Mustelus* when he found the

place of attachment of the hypophyseal stalk near the anterior end. In a 90 mm. *Mustelus* this place of attachment can be recognized by the very thin floor, and in the adult, by the opening into the subdural space (Haller '96, figs. 12 and 40).

The cavity in the hypophysis of elasmobranchs has been variously described as barely distinguishable, slit-like and large. It is large in some adult specimens of *Acanthias*. In the anterior lobe there is a distinct increase in the size of the cavity during its development. Table 2 will show the actual increase in the depth of the cavity. The measurements here given were taken in the caudal part of the anterior extremity of the anterior lobe.

TABLE 2
Showing depth of the hypophyseal cavity

SPECIMEN	THICKNESS OF ROOF IN MICRA	DEPTH OF CAVITY IN MICRA	THICKNESS OF FLOOR IN MICRA
Embryo 34 mm.....	31	37	25
50 mm.....	31	13	18
86 mm.....	35	12	22
Pup.....	56	90	50 including glandular outgrowths
Adult.....	56	470	480 including glandular outgrowths

The increase in size of the lumen from the pup to the adult is thus seen to be considerable. From the table and from comparison with figures 2 to 10 it is seen that the increase in size of the lumen is not gradual through all the stages. For example, in the 50 mm. embryo the lumen is actually smaller than in a 34 mm. embryo. It is only from the early pup stage on that the lumen increases to any considerable degree. The increase in size of the lumen of the inferior lobes is even more marked. The cavity in the superior lobe is never large. It is very small in a 48 mm. embryo where the lateral wings are first prominent. In a 95 mm. embryo this cavity is small, but is still distinct. The lumen of the middle portion of the superior lobe extends transversely and forward in the lateral wings of the superior

lobe in pups. There is no evidence at any time of an extension of the lumen into any of the glandular columns of this part. In the adult all trace of a central cavity has disappeared, unless the small secretion spaces, to be described later, are remains of the original lumen. Occasionally there is a more or less prominent median dorsal extension of the cavity of the anterior into the superior lobe. In a very few cases this dorsally-extending cavity is continuous with a secretion space lying laterally.

Table 3 shows the increase in size of the hypophysis; also, the length of the hypophysis at the median line, the greatest

TABLE 3
Showing increase in size of the hypophysis

SIZE OF SPECIMEN	LENGTH OF HYPOPHYSIS IN MM.	GREATEST WIDTH OF SUPERIOR LOBE IN MM.	GREATEST WIDTH OF ANTERIOR EXTREMITY IN MM.
Embryo 22 mm.	0.69		0.23
28 mm.	0.81	0.40	0.22
33 mm.....	1.04	0.96	0.30
48 mm.....	1.00	1.07	0.35
95 mm.....	1.34	1.30	0.40
Pup.....	3.54	1.91	0.53
Adult.....	6.00	4.00	1.00

width of the anterior extremity of the anterior lobe, and the greatest width of the superior lobe. The table shows there has been a constant increase in length which is rather more rapid in earlier stages. The increase in width of the superior lobe is a gradual one in the beginning. The anterior lobe, however, grows very little at first. This part doubles in width in its growth between the 22 mm. and the pup stages. It almost doubles again in its later growth.

From a brief study of a few *Torpedo* embryos, I am inclined to believe, with Sterzi, that the lateral lobes, described in this form by Gentes ('08) and others, are comparable with the inferior lobes of *Acanthias*. The embryos examined show no prehypophyseal body such as Chiarugi ('98) described in this genus, nor is there anything comparable to the prehypophyseal body found in the embryos or adults of *Acanthias*.

HISTOLOGY AND HISTOGENESIS

1. *Histology of the adult hypophysis*

a. Anterior lobe. The deep median sulcus in the floor of the anterior lobe has been noted above. It has been observed by Sterzi and others in various selachians. The furrows noted in the floor of the anterior extremity in the pup are evidence of the beginning formation of tubules. Haller observed the tubules on the ventral wall of the anterior part in *Mustelus*, Gentes observed them in *Torpedo*, and Sterzi in *Acanthias*. Tilney ('11) stated that there are many vesicles in the upper and lower walls of the juxta-neural (anterior) part in *Acanthias*. Haller figures cyst-like glands in the roof of the anterior lobe of *Mustelus*. Some adult specimens of *Acanthias* show glandular outgrowths in the superior wall of the anterior lobe, especially in the dorso-lateral parts of the anterior extremity. As Sterzi ('09) observed, glands probably develop from the anterior lobe throughout life and, finally, even from the dorso-lateral walls and roof, and glands project ventrally from the floor of the anterior lobe. A model of some of the latter shows that branches are given off at all angles from the first large tubule extending ventrally and that secondary and tertiary outpouchings occur from the branches. The tubules anastomose among each other and with those from other glands both cranially and caudally, but not across the median sulcus with the glands of the other side. The lumina may be continuous through the anastomosing tubules. A cast of the lumina shows frequent enlarged cavities from which small openings lead to the cyst-like cavities found in some of the secondary and tertiary tubules. No such glands were observed in any other part of the anterior lobe. If any glands be present in the roof of the anterior end they are simple and cystic or acinar in character. In some specimens, probably older animals, there are large tubular glands in the floor and lateral walls and even in the roof of the caudal extremity. The floor of the middle part occasionally shows cystic glands. The walls of the tubules are two or three cells thick and the columnar cells forming them are at right angles to the surface (fig. 28). There is a periph-



Fig. 28 Transverse section of the floor and a gland of the anterior lobe of an adult. $\times 400$. s, sinusoid.

eral zone of cytoplasm which is very finely granular and contrary to the statement of Tilney, it is acidophilic. Haller observed that the peripheral zone of cytoplasm in *Mustelus* stained intensively with borax carmine and Stendell stated that the peripheral layer of cells is acidophilic. Sterzi ('09) found that the cytoplasm of the cells in the floor of the caudal part of the rostral lobe is not granular. However, there are glandular outgrowths here in some adults and these walls have the same character as the other glandular parts, except in the mid-ventral line where no outgrowths are found, and here the wall is composed of low epithelial cells. The nuclei are oval and crowded nearer the inner free surface. They have a finely granular chromatic network. The roof of the anterior lobe is also composed of several layers of cells. If no glands are present, the nuclei here are very irregularly placed, some of them lying parallel to the surface along the inner free side, others being placed at various angles to the surfaces. The cytoplasm is very scant. The roof comes into close relation with the overlying brain, but a thin connective tissue layer containing small blood vessels and capillaries separates them. One cannot speak of a fusion of the roof of this lobe with the brain tissue above, as noted by Tilney.

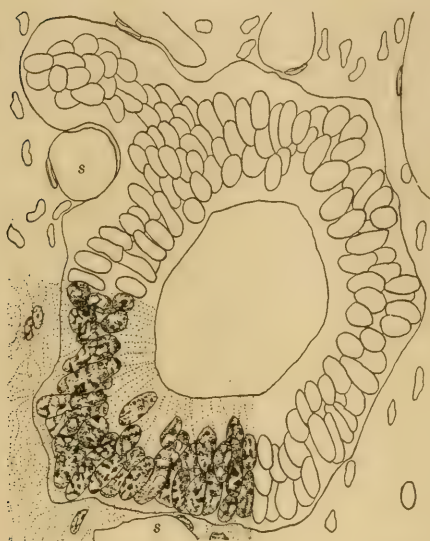


Fig. 29 Transverse section of a gland of the inferior lobe of an adult. $\times 400$.

b. Inferior lobes. The inferior lobes are large glandular structures, from the walls of which are many tubular outgrowths. These tubules are especially numerous on the ventral surfaces of the inferior lobes although there are some on the roof also. Two or three layers of columnar cells form the walls. In this case a wider cytoplasmic zone lies along the inner free surface (fig. 29). The cytoplasm is clear and the cell membranes stand out distinctly. The cells are very faintly acidophilic. The nuclei here, as in the anterior lobe, are oval in outline, and the long axis is always at right angles to the free surfaces. The chromatin here also is in a fine network with a more or less definite layer along the nuclear membrane.

c. Superior lobe. As has been observed in all selachians, there is a great glandular outgrowth from the roof of the superior lobe. As Sterzi has noted, these glands are not tubular but solid. The floor of this portion shows no glandular outgrowth. It is made up of several layers of columnar cells. The roof is several cell-layers deep and from it many thick columns extend upward.



Fig. 30 Sagittal section of the glandular cords of the superior lobe of an adult. $\times 400$.

The nuclei of the columns are spherical in shape, crowded close together in the center, and contain a light chromatin network and a nucleolus. Between these columns are numerous sinusoids. Along the periphery of the columns is a thick layer of granular cytoplasm, the granules being small and closely crowded (fig. 30). The cytoplasm frequently takes a distinctly acid stain. In some eosin-methylene blue preparations the cytoplasm of the superior lobe along the periphery of the columns is stained blue. With iron hematoxylin this same zone sometimes retains the hematoxylin longer than does the chromatin. The granules of this part often stain an intense blue with Mallory's phosphotungstic acid stain. On either side of a granular area which stains very deeply there may be a clear area where the secretion possibly may be forming or has just been given up. I cannot, therefore, agree with Tilney ('11) who finds only eosinophilic cells in the superior lobe and basophilic in the anterior lobe. Besides, as has been noted, in many cases the cells of the anterior lobe take eosin quite as readily. Stendell ('13) thought that the division of chromophobic and chromophilic portions as described by Sterzi and others might not be true in all cases. In the adult *Acanthias*, it seems to me this distinction cannot be sharply drawn, at least not with all stains. The cytoplasm of the supe-

rior lobe stains readily, and indeed, sometimes more deeply than that of the anterior lobe. The nuclei, on the other hand stain less deeply than those of the anterior lobe. Those of the inferior lobe frequently stain very lightly. Sterzi has stated that the cytoplasm of the superior lobe stains with difficulty.

d. Connective tissues, blood vessels and nerves. In very small embryos there are only occasional connective tissue cells lying between the hypophysis and the brain and vascular sac above, and these cells are along the dorso-lateral walls. Beginning in 33 mm. embryos, however, there is a thin layer of mesenchyma between the dorsal (posterior) end of the hypophysis and the anlage of the vascular sac. In 50 mm. embryos small blood vessels are found between the superior lobe and the saccus vasculosus (fig. 37). At this time, also, a thin layer of mesenchyma separates the hypophysis from the brain floor. In the superior lobe of the adult occasional small strands of connective tissue are found in the center of the core of nuclei of the columns.

They anastomose with the connective tissue between the columns but have no cytoplasmic zone bordering them as do the larger, more vascular connective tissue strands between the columns.

In the pup, there are small capillaries in the connective tissue over the anterior lobe of the hypophysis. On the ventral side there is considerable connective tissue between the developing tubules. The few capillaries here are not large. The capillaries between the columns of the dorsal lobe are large and numerous. These capillaries or sinusoids are to be found in the interstices between the cell columns and also between the ends of the columns and the overlying vascular sac. In the adult the capillaries over the anterior lobe are somewhat larger. There has been some increase in the size and number of the capillaries between the tubules of the anterior lobe and the same is true in the superior lobe between the cell columns.

Nerves have been described in the hypophysis by Edinger ('92), Sterzi ('09) and others. Sterzi described the floor of the brain in the hypophyseal area (above the superior lobe of the hy-

pophysis) as composed of three layers, of which the middle was made up of nerve fibers coming from the caudal end of the inferior lobes of the brain. When these fibers reach the area above the superior lobe, numerous bundles of them go ventrally between the columns. These bundles are large and composed of large nerve fibers. According to Stendell ('12) a distinct lumen, continuous with the lumen of the vascular sac, extends into these bundles in *Heptanchus*. As Sterzi ('09) showed, these bundles are solid in *Acanthias*. Sterzi was not able to see any of the fibers ending in the cell columns. In some material stained with Mallory's phosphotungstic acid hematoxylin these fibers are well shown. I can not affirm that they do end in the cell columns between the cells as Sterzi was inclined to believe.

e. Secretions. Haller ('96) stated that the lumina of the superior lobe may contain cell detritus or a secretion. Tilney ('11) observed a colloid-like substance in the vascular sac above the superior lobe. Stendell ('13) described deep acidophilic secretion granules in the cytoplasm of the cells of the 'Zwischenlappen.' These colloid-like secretions were found in the cells lying towards the blood vessels. The adult *Acanthias* studied show no colloid-like secretion granules, such as Stendell found in the cytoplasm near the sinusoids in *Mustelus* and *Scyllium*. Considerable colloid-like secretion, however, is found in the tubules of the anterior lobe, also some in the main lumen of this part. The tubule drawn in figure 28 contains secretion. Some secretion was found in the tubular glands and in the main lumen of the inferior lobes. There are also many spaces in the superior lobe which are partially filled with secretion. The spaces are cylindrical in shape, sometimes as much as 20μ in diameter and 50 to 200μ long. They have no special walls, the cells and nuclei lining them appear to have been crowded aside. Frequently the inner layer of nuclei lies flat along the wall. These spaces never come in contact with the sinusoids, but are always found in the middle of the columns and are surrounded by nuclei which are crowded close together. Aresu (14) has described similar cyst-like spaces in *Chimaera*, containing a substance which stains lightly with basic stains. That the secretion does not fill the

spaces may be due to shrinkage, as has been suggested in the case of the colloid in the thyreoid follicles. In all respects it is similar to that found in the anterior lobe. There is no direct outlet by means of which a secretion can reach the cavity of the vascular sac and thus gain entrance to the ventricles of the brain. The absence of any secretion in the vascular sac also argues against there being a pathway for the secretion to enter it. It would seem probable that another secretion, distinct from this, is formed in the lumen of the tubules. As stated in the description of the histology of the adult hypophysis, the granular cytoplasm is always found on the peripheral side of the cell cords or tubules and never on the side toward the lumen. It is probable that this secretion finds its way into the numerous capillaries along the periphery. In the formation of two secretions, therefore, the hypophysis resembles the thyreoid in some forms, and also the hypophysis in some mammals.

2. *Histogenesis of the hypophysis*

In 7.5 mm. embryos the walls are formed of a single layer of cells of a large cuboidal type. The cytoplasm is slightly granular and somewhat acidophilic. The nuclei are large, somewhat oval, placed near the basement membrane, and contain considerable chromatin network. Usually several nucleoli, although sometimes only one, are found near the nuclear membrane (fig. 31). Very soon the walls of the hypophysis are composed of several layers of cells. In a 13 mm. embryo the large nucleoli are no longer so prominent and, as a rule, only smaller pseudonucleoli, as observed by Sterzi, are to be seen.

a. Anterior lobe. In a 21 mm. embryo, very little of the floor of the anterior lobe is as yet present. The walls are composed of two layers of columnar cells which have a thin outer and a thick inner, slightly granular cytoplasmic zone. The large and elongately oval nuclei contain a dense chromatin network, especially in the anterior end (fig. 32). Sterzi has observed that there are some elongated nuclei in the roof of the hypophysis in which the long axes are at right angles to the surfaces, and

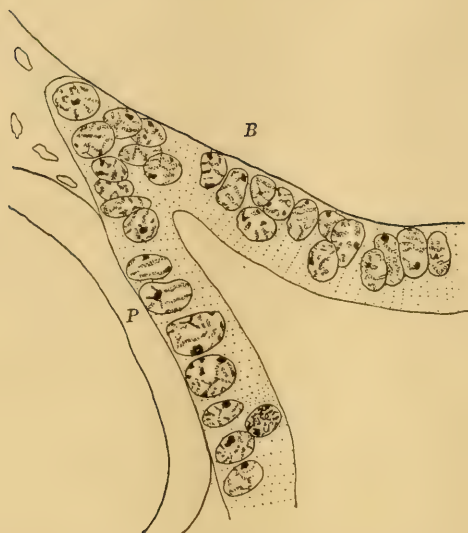


Fig. 31 Transverse section of a portion of the hypophyseal anlage of a 7.5 mm. embryo. (H.E.C. 1503). $\times 500$. *B*, region of brain; *P*, premandibular somite.

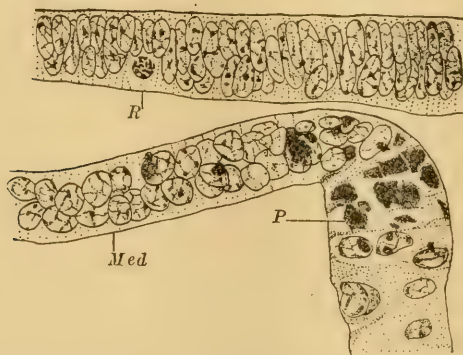


Fig. 32 Sagittal section of the roof of the anterior lobe in a 21 mm. embryo. (H.E.C. 1493). $\times 450$. *R*, roof of anterior lobe; *Med*, medial connection of inferior lobes; *P*, pigment granules.



Fig. 33 Sagittal section of the floor of the anterior end and of one of the tubules of the anterior lobe of a pup. $\times 400$.

between these are spherical shaped nuclei. He has described these in the roof of the superior lobe. These become more evident in 40 and 50 mm. embryos. In an 86 mm. embryo, the nuclei of the roof are to some extent irregular in arrangement. The cytoplasm takes orange G quite readily and hence is somewhat acidophilic. In the pup, the floor is well formed and is now the thicker wall of the anterior lobe. As shown in figure 33, it is composed of four or five layers of nuclei. The oval nuclei are crowded close to the inner free surface. There is, however, a narrow outer cytoplasmic zone which is very granular. The character of the cells of the glandular outgrowths is shown in figure 33. Here, too, there is a narrower outer rim of granular cytoplasm. A wider inner zone of non-granular cytoplasm bounds the lumina of the tubules. The nuclei, as in the floor, are oval. Usually two or three layers form the walls. The nuclei have a distinct chromatin network with occasional denser-staining chromatin masses resembling nucleoli.

b. Inferior lobes. The development of the inferior lobes as constrictions of the lateral walls of the early hypophyseal outpouching has been described above. The point where these lobes will grow together across the median line is indicated in figures 5 to 10. The character of the cells forming this part differs from the rest of the hypophyseal outpouching as early as the 21 mm. stage (fig. 32). The cytoplasm here stains less densely than that of the rest of the hypophysis. The nuclei are distinctly spherical in shape and have a very scant chromatin network. A part of this floor, immediately posterior to the hypophyseal stalk, contains a considerable amount of a granular yellowish pigment. Both Müller ('71) and Hoffmann ('96) have noted the



Fig. 34 Sagittal section of the floor of the hypophysis near the median plane, showing the part which connects the inferior lobes. (H.E.C. 362). $\times 400$.

pigment in the stalk of the hypophysis. The nuclei become more oval in older embryos. This is well shown in figure 34 which is a mid-sagittal section of the region which later forms the connection between the inferior sacs. The nuclei here are very irregularly placed. Extending caudally from the stalk are several nuclei flattened along the inner free surface. The cytoplasm stains very lightly. The pigment masses are numerous. Caudally there is a sudden transition to columnar cells of the kind found in the wall of the anterior lobe. The floor in this region is as just described until the 48 to 50 mm. stages when the inferior sacs are completely constricted from the anterior one. The pigment and the flattened nuclei are found only near the median line. Both are still present in a 41 mm. embryo.

In the inferior sacs proper, however, which are formed at the lateral sides, the cells are similar to those of the floor of the anterior lobe. The outer, narrower zone of cytoplasm, as well

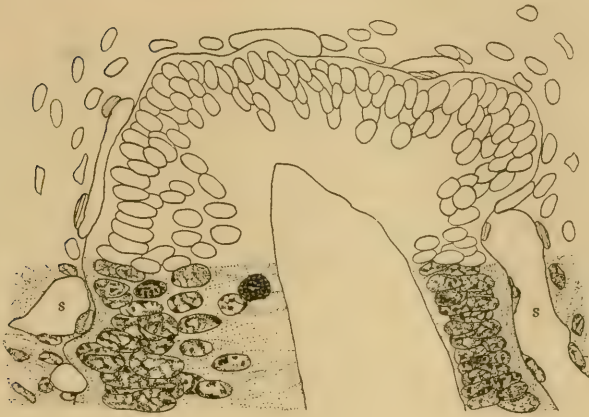


Fig. 35 Sagittal section of a portion of the inferior lobe of the hypophysis of a pup. $\times 400$.

as the inner, wider one, stains lightly in a 50 mm. embryo. In an 86 mm. embryo the outer zone has fine granules. The cells are slightly acidophilic. The nuclei are elongately oval in outline as in the anterior lobe. In the pup many outpouchings indicate the beginning glandular development. The upper wall is three or four cell-layers thick (fig. 35). There is a wide, inner, clear-staining cytoplasmic zone. The outer narrower rim is slightly granular. The nuclei are oval in outline, as in the anterior lobe. Numerous densely staining chromatin masses are to be seen. The lower wall or floor is much thinner. It is composed of only one or two layers of cells and has a narrow, inner, cytoplasmic rim. This zone, as in the roof, stains very lightly and is non-granular. The outer rim is, however, quite granular. The nuclei are like those in the roof but contain a somewhat denser chromatin network.

c. Superior lobe. In a 21 mm. embryo the wall of the superior end of the hypophyseal anlage is thickest where the superior lobe later develops (fig. 36). The nuclei are large and oval and have a light chromatin network. In a 50 mm. embryo (fig. 37) the wall in this region is considerably thicker than in the 21 mm. embryo because of an increase in the number of cell layers. There



Fig. 36 Sagittal section of the superior end of the hypophysis of a 21 mm. embryo. (H.E.C. 1493). $\times 450$.

Fig. 37 Sagittal section of the superior lobe of a 50 mm. embryo. (H.E.C. Series 444). $\times 450$.

is an outer cytoplasmic zone which is non-granular and stains lightly. The nuclei are smaller than in the 21 mm. embryo and contain less chromatin. As Sterzi has stated, there are some spherical nuclei and some more slender oval nuclei, but no regular arrangement of these, such as Sterzi described, was observed. In a 95 mm. embryo, the roof of the superior lobe has increased in thickness (fig. 38). The nuclei are oval and contain a denser network of chromatin than is found in 50 mm. embryos. Along the periphery many of the nuclei are spherical. There is an inner zone of cytoplasm as in the 50 and 21 mm. stages, which is

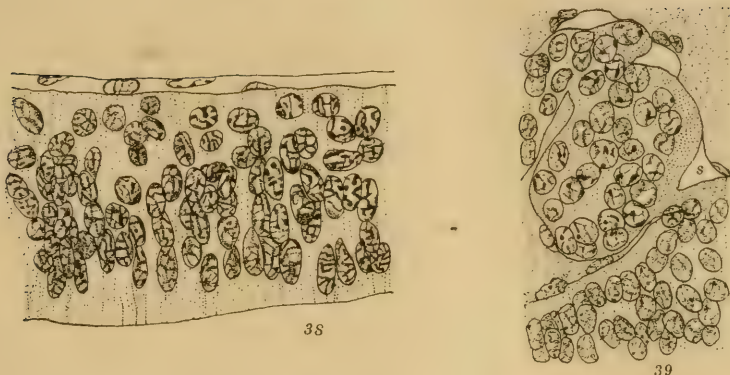


Fig. 38 Transverse section of the superior lobe of a 95 mm. embryo. (H.E.C. Series 1882). $\times 450$.

Fig. 39 Sagittal section of the superior lobe of the hypophysis of a pup showing one of the cell columns and a portion of the roof. $\times 400$.

non-granular. Fewer mitotic figures are to be found at this time. In the pup the roof is a little thicker than in the preceding stage. Above the roof there are many cell columns which are outgrowths from the roof proper (fig. 39). The relations of cell columns and roof to each other are shown in figure 39. The roof has a narrow outer zone of granular cytoplasm. Its nuclei are oval and have a light chromatin network. The cell columns come into close contact with the overlying floor of the vascular sac (fig. 39). Numerous capillaries and a loose connective tissue fill the spaces between the cell columns and between the roof of the hypophysis and the floor of the vascular sac. The columns have an outer, granular cytoplasmic zone. The cells are acidophilic. The nuclei are spherical in outline, have a light chromatin network and usually one or two larger chromatin masses or nucleoli.

Only a brief statement can be given at this time concerning the development of the glandular columns of the superior lobe. In the region of the superior lobe in all embryos up to the 50 mm. stage, the nuclei are elongate-oval in outline and are arranged in two or three layers (fig. 36). In a 50 mm. embryo some of the nuclei at the periphery are spherical (fig. 37). It is possible

that the elongate-oval nuclei in this region in the younger embryos are changed into spherical ones. In the pup there are numerous columns extending dorsally, which consist of a central group of spherical, light-staining nuclei and a peripheral zone of cytoplasm. Until some material between the 95 mm. embryos and the pup stage is studied, it must remain a question how these cell columns are formed. It is possible that the cells with spherical nuclei arrange themselves in groups and these groups then evaginate from the roof. The scarcity of these groups in the 95 mm. embryos and in all late embryonic stages argues against such a possibility, although Sterzi's observations on the presence of groups of spherical nuclei lying between masses of cells with oval nuclei should be taken into consideration. Numerous cyst-like outpouchings are present in some adults in the anterior part of the superior lobe, or, rather, between this and the roof of the caudal extremity of the anterior lobe. Some of these outpouchings show areas of cells similar to those forming the columns of the superior lobe, interspersed with areas of cells like those of the anterior lobe. The areas of cells resembling those of the superior lobe may form the entire wall or may lie on a basement of cells resembling those forming the anterior lobe which line the cavity. This would indicate that the regions of the anterior and superior lobes are not sharply separated, or, that the cells of this region which still resemble the embryonic condition change into cell columns of the superior lobe. This need not imply, however, that the cells of the anterior and inferior lobes are of a more embryonic type, although they may be more primitive phylogenetically.

3. Development of the interhypophyseal canal

The formation of the ridge connecting the inferior lobes on the dorsal (posterior) side of the hypophyseal anlage had been described. The character of the epithelium in this region in a 21 mm. embryo, as stated above, differs from that of other parts. A ridge is prominent in a 34 mm. embryo. In a 40 mm. embryo the groove on the inside of this ridge is marked (fig. 8). In a

48 mm. embryo the ridge is very distinct and the connection of the lumen of this part with the lumen of the anterior lobe has become constricted. The constriction forms the narrowed connection of the inferior sacs to the anterior lobe. As previously stated, the growth of the furrows separating the inferior sacs from the lateral sides of the anterior lobe is well marked at this time. A sagittal section of the hypophysis of a 50 mm. embryo shows a short interhypophyseal canal (fig. 9). In a 95 mm. embryo the canal has lengthened considerably. The walls are composed of one or two layers of low columnar cells. In the pup the canal is longer than in the embryos but the diameter is about the same as in younger specimens. In the adult the canal has increased in length and diameter and is attached in the floor of the anterior

TABLE 4

Showing the size of the interhypophyseal canal

SIZE OF SPECIMEN	LENGTH OF CANAL IN MM.	DIAMETER OF CANAL IN MM.
Embryo of 50 mm.....	0.13	0.042
95 mm.....	0.36	0.048
Pup.....	0.44	0.048
Adult.....	0.68	0.080

lobe at or near its caudal end. Its other attachment is near the dorsal side of the connection between the inferior lobes. There are no tubular outgrowths from it such as Haller found in *Mustelus*. Table 4 shows the size of the canals at different stages. It will be seen that there is a continual increase in the length of the canal. The diameter in the older specimen is somewhat greater than in the 50 mm. embryo, although there is no great change. A distinct lumen is present. In the median line there is a well defined layer of connective tissue extending from the tip of the parachordal plate (fig. 9) forward to the floor of the anterior lobe of the hypophysis. This layer develops rapidly after the canal is formed and surrounds it. It is well defined in a 50 mm. embryo, but becomes thicker in the adult. This layer separates the inferior lobes from the rest of the hypophysis and

makes their dissection difficult. The cells and fibers in it are arranged concentrically around the canal. In the adult, the layer of connective tissue is still prominent and extends the entire length of the canal.



Fig. 40 Transverse section of the hypophyseal stalk of a 28 mm. embryo. (H.E.C. Series 1357). $\times 350$.

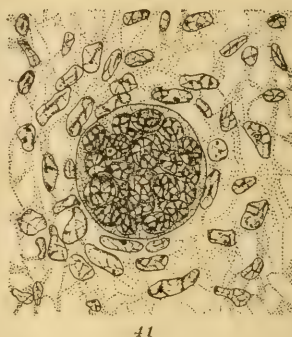


Fig. 41 Transverse section of the hypophyseal stalk of a 33 mm. embryo. (H.E.C. Series 307). $\times 350$.

4. *Development of the hypophyseal stalk*

The stalk connecting the hypophysis with the mouth is formed in 22 to 24 mm. embryos. It is present in one 21 mm. embryo which shows the anterior end constricting from the mouth. In these stages it is oval in cross-section, the lumen is very large, and its walls are formed of a layer of low columnar cells, the nuclei of which are somewhat elongated and contain considerable chromatin. In the posterior (superior) margin are found many yellow pigment granules, as has been described by Hoffmann ('86). These granules are found within the cell, sometimes apparently in the nucleus (fig. 32). As Hoffmann stated, they are first seen in the bucco-pharyngeal membrane, but later occur also in the wall of the stalk. Müller ('71) had described them in the stalk in *Acanthias* embryos of 30 mm. length.

In 28 mm. embryos the lumen in the stalk is small. The wall consists of a double layer of epithelial cells, the outer of which

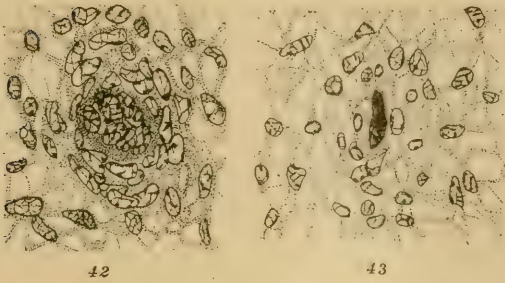


Fig. 42 Transverse section of the hypophyseal stalk of a 37 mm. embryo. (H.E.C. Series 363). $\times 350$.

Fig. 43 Transverse section of the hypophyseal stalk of a 41.5 mm. embryo. (H.E.C. Series 369). $\times 350$.

is low columnar in type (fig. 40); the inner is irregular and the nuclei are oval in outline but not regularly placed. Figure 40 is of a section through approximately the middle of the stalk. The lumen and stalk are larger where it connects with the hypophysis and with the mouth, both ends being funnel-shaped and joined by a narrower middle part. The connective tissue around the stalk is mesenchymal in character. Immediately around the stalk the cells are concentrically arranged. This character is more pronounced in later stages.

In a 33 mm. embryo the two ends of the stalk are funnel-shaped, as before, and contain a lumen, while the middle part of the stalk is made up of a mass of cells in which no lumen is present (fig. 41). The outer layer of cells, though still definite at the ends, is no longer so in the middle part. The nuclei are more spherical in shape and contain a denser chromatin network. The arrangement of the mesenchymal cells around the stalk is concentric.

In a 37 mm. embryo the stalk is greatly reduced in size. The nuclei are massed in the center and are surrounded by a densely staining cytoplasm (fig. 42). Some pigment is scattered throughout the stalk, as in all of the specimens. The concentric arrangement of connective tissue cells is more marked.

In some embryos 40 mm. in length no remnant of the stalk is found. In a 41.5 mm. embryo a small elongated densely stain-

ing stalk is present (fig. 43). What apparently is the remains of the nuclear mass is surrounded by a narrow densely-staining cytoplasmic rim. The chromatin network has disappeared, but larger masses of densely staining chromatin are to be seen. The concentric arrangement of the connective tissue cells is apparent, but not so marked as in the younger stages. In another specimen, 40 mm. in length, a strand of connective tissue, extending from a funnel-shaped mass of epithelial cells—continuous with the epithelial lining of the mouth—to the base of the hypophysis, indicates the position of the degenerated stalk (fig. 8). The hypophyseal attachment of the stalk is anterior to the groove connecting the inferior lobes. Soon after this time the cartilages at the base of the brain become continuous across the mid line.

I wish to thank Dr. R. E. Scammon for his many helpful suggestions throughout this work. Thanks are also due to Dr. R. J. Terry for his kindly interest during its completion.

SUMMARY

1. The terms 'anterior lobe,' 'inferior lobes' and 'superior lobè' have been used for the several parts of the hypophysis of *Acanthias*.

2. Rathke's pouch forms the posterior part of the anterior lobe. The later evagination of the ectoderm, anterior to this, forms the middle portion and the anterior extremity of the anterior lobe.

3. The inferior lobes develop from the lateral sides of the posterior extremity of the anterior lobe, i.e., from the lateral sides of Rathke's pouch.

4. The superior lobe develops from the caudal (superior) end of the hypophyseal anlage.

5. In the course of development the hypophysis shifts in position about 145 degrees, so that the upper wall becomes the floor and the ventral (anterior) surface the roof.

6. There is glandular growth from the roof of the superior lobe and the inferior lobes, as has been described by Sterzi and others, and from the floor of only the anterior and posterior extremity of the anterior lobe in all adults.

7. The cells of both anterior and inferior lobes are acidophilic in character.

8. The cell columns of the superior lobe are solid as Sterzi described them.

9. Frequently the anterior and inferior lobes stain more densely than does the superior lobe. In these cases, it is the nuclei which take the darker stain. In general the anterior and inferior lobes may be considered the chromophilic ones.

10. Spaces containing some colloid-like secretion are present in the superior lobe. A similar secretion is present in the lumina of the tubules of the anterior and inferior lobes and in the large main lumen.

ADDENDUM

After the completion of the present work a paper by M. W. Woerdeman: "Vergleichende Ontogenie der Hypophysis" appeared (Arch. f. mikr. Anat., Bd. 86). This investigator figured Rathke's pouch in an 8 mm. *Torpedo* embryo. In somewhat older embryos (12-15 mm.) the region where Rathke's pouch opens into the mouth evaginates and in still later embryos a region anterior to this is constricted from the mouth. The hypophysis then consists of a small Rathke's pouch somewhat constricted from an anterior (ventral) 'Mittelraum' and anterior to the latter, the 'Vorraum.' The middle division, in 20 mm. embryos, divides by a circular constriction into a dorsal and a ventral part. In this way the ventral sacs are formed. The hypophyseal stalk now opens into the ventral sacs, in which observation Woerdeman agrees with that of Gentes and Stendell. According to Woerdemann's comparison of the parts of the hypophysis with those described by Stendell, Rathke's pouch is homologous with the superior lobe (table 1, p. 400) and the 'Mittelraum' and 'Vorraum' are homologous with the anterior lobe. The ventral sacs and lateral lobuli which he described are probably homologous with the inferior lobes. In *Squalus* I have described Rathke's pouch, or the early anlage of the hypophysis as giving rise to the caudal extremity of the anterior lobe. A later evagination ventral to this gives rise to the middle portion and anterior extremity of the anterior lobe. The secondary evagination is early recognized, as the epithelium here is thickened (page 408). The opening from the mouth to the early hypophyseal anlage, or Rathke's pouch, secondarily comes to open in the later evagination (page 410) of which there is only one in *Squalus*. The inferior lobes and the superior lobe develop from the early hypophyseal anlage in *Squalus*, as has been described (pp. 410-11). The hypophyseal stalk is not constricted from the anterior lobe with the developing ventral lobes but remains connected with the caudal wall of the middle portion of the anterior lobe until it disappears.

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THE ANATOMY OF HETERODONTUS FRANCISCI

II. THE ENDOSKELETON¹

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THIRTY-ONE FIGURES (EIGHT PLATES)

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INTRODUCTION

The skeleton of the elasmobranch fishes has served for numerous investigations which have contributed much to our knowledge. Principal among these researches may be mentioned 'Das Kopfskelet' of Gegenbaur ('72) dealing with the head; studies on the

¹ Part I, The exoskeleton, published in Univ. Calif. Pub., Zool., 1914, vol. 13, p. 147.

column by Hasse ('79); and on the anatomy and development of the fins by Thacher ('77); by Mivart ('79), by Gegenbaur ('65), and by Balfour ('81). From exhaustive studies of the first group we have corrected our understanding of the nature of the skull, while from those of the third we have gained illuminating evidence as to the origin of paired limbs.

While considerable attention has been given to parts of the skeleton of the heterodont sharks, yet, so far as I am aware, no approximately complete study of any member of this group has previously been made. This may be due, at least in part, to the fact that the heterodont sharks are located in widely separated regions, and, furthermore, that they are not abundant in the localities where they occur. *Heterodontus* is more or less difficult to obtain on the California coast, yet through the efforts of the Scripps Institution of the University of California I have been provided with numerous specimens of various ages. I wish here to re-express my thanks to that Institution for its assistance.

While the head region of *Heterodontus* is specialized, as Gegenbaur maintained, yet it is by no means without generalization. In fact marked simplicity may be found side by side with specialization. Such a case is seen in the generalized type of quadrato-mandibular joint, accompanied by a highly specialized mode of ligamentous articulation.

It would be generally conceded that the fin skeleton also is specialized, if one agree with Mivart's conception of specialization meaning concrescence. However this may be, it is certain that, had Mivart known of the unpaired fins of *Heterodontus francisci*, his argument for similarity of plan between paired and unpaired fins would have been even more convincing.

THE ENDOSKELETON

In the following paper I shall discuss at some length the endoskeleton of *Heterodontus francisci*. In such a consideration the skeleton naturally is divided into: (1) The axial part, including the skull and spinal column; and (2) the appendicular skeleton, embracing fins and fin-girdles.

I. AXIAL SKELETON

1. *The skull*

The skull, like that of elasmobranchs in general, is composed of: (a) A cranium or brain case to which the sense capsules are fused in the adult; and (b) a series of cartilaginous visceral arches which support the buccal and pharyngeal regions.

a. The cranium or brain case in dorsal view (fig. 1) is roughly quadrilateral in shape, slightly bifurcated at the anterior and posterior margins, and constricted along the sides at the first and second thirds—the first of these indentations being much the more pronounced. The cranium is a closed box except at the antero-dorsal end, where there is a large opening, the anterior fontanelle (*F.*) and at the posterior end, where is located the foramen magnum (*f.m.*) through which the spinal cord joins the brain.

In the mid-dorsal line joining these two openings several structures appear. These are, passing forward, a ridge, the occipital crest (*o.cr.*) which runs to the parietal fossa (*p.f.*); from the bottom of the latter the endolymphatic ducts lead to the ears. In front of this pit is a slight elevation which sinks immediately into a long groove—the parieto-frontal groove (*p-f.g.*) which, in turn, broadens out into the anterior fontanelle.

On each side of and running parallel to the parieto-frontal groove there is a row of foramina extending posteriorly to the level of the parietal fossa. Anteriorly the first two of these on each side are the ophthalmics, through the first of which passes the ophthalmicus profundus nerve (*f.o.p.'*), through the second, the ophthalmic division of the seventh nerve (*f.o.VII'*). Through the numerous and smaller perforations which follow pass branches of nerves, and through the succeeding large foramina, blood vessels. These openings terminate posteriorly at an elevation produced by the anterior oblique semicircular canal (*a.o.s.*) which with a similar elevation from the opposite side roughly forms a broad V enclosing at its apex the parietal fossa. Below this there is a large inverted lower V, the arms of which enclose the foramen magnum (*f.m.*), and the apex of which abuts

against that of the V above described so that the two V's roughly form an hour-glass.

Viewed from the ventral side (fig. 2) the cranium is roughly flat-iron-shaped, with the apex projecting between the olfactory capsules (*ol.c.*). At the most posterior part of the cranium is a niche, the sides of which are produced by the occipital condyles (*o.cd.*). In the mid-ventral line, one-fourth the distance from the posterior border to the tip of the nose, is a foramen (or a pair of foramina) through which the internal (posterior) carotid arteries reach the brain (*f.i.c.*); laterad of these on each side are similar perforations (*f.e.c.*) through which the external (anterior) carotids pass on their way to the orbital region. A line through the internal carotid foramina, and at right angles to the long axis of the cranium, divides the ventral cartilaginous mass into two regions, the anterior of which is the embryonic trabecular region, the posterior region that of the parachordal cartilages.

Along the lateral margin, in ventral view, from behind forward are the post-articular processes (*po.hm.*) bounding posteriorly the deep fossa into which the hyomandibular cartilage fits. Anterior to the fossa is a pre-articular process (*pr.hm.*). In front of the latter is a constriction, anterior to which is a wide projection—the basal plate (*b.p.*); considerably in front of the basal plate, at the sides, is the palatal fossa (*pl.f.*) into which a projection of the palatoquadrate cartilage fits. At the anterior tip of the cranium the basitrabecular cartilage (*b.tr.*) arches upward to be met by two dorsolateral rostral pieces coming down from the dorsal part of the cranium. At the sides of the basitrabecular piece are the external openings for the olfactory capsules (*ol.c.*).

In this view may be described the olfactory capsules and the nasal cartilages at their margins. The capsules are thin, cartilaginous structures which are formed as the skeletogenous protection for the olfactory organ. Dorsally the capsules are continuous with the cranium (see also fig. 1); ventrally they thin out to delicate lamellae of cartilage which surround the nasal aperture, excepting in the postero-medial part, where the

wall is membranous (fig. 2). The olfactory cup or inside of the capsule communicates internally with the cranial cavity by the olfactory foramen, through which the first cranial nerve passes.

At the free margin of the capsule there is a scroll-like nasal cartilage (*n.c.1*, fig. 2), which runs on the outer margin around the aperture of the capsule. Both the anterior and posterior ends of the cartilage recurve upon themselves medially so as to form a narrow ellipse, across which, from its antero-lateral third, a projection extends backward and inward, forming of the ellipse a figure 8.

A second nodule of cartilage (*n.c.2*; not figured by Gegenbaur '72 for *H. philippi*, pl. 16, fig. 2) is loosely attached to the anterior end of the first nasal cartilage. The attachment is made at its broader anterior end and its free tip extends backward to be connected by tissue with the deeper recurved anterior end of the first cartilage.

In side view (fig. 3) the olfactory capsules occupy a position remote from the main part of the cranium. Projecting from the postero-lateral part of the cranium are the thick-walled auditory capsules (*a.c.*) which give protection to the organs of hearing. Between the auditory and nasal capsules is the large socket or orbit in which is located the eye. Overhanging the orbit is the broad supraorbital crest (*s.o.*) from the anterior part of which arises the preorbital process (*pr.o.*) and from the posterior, the post-orbital process (*po.o.*). The floor of the orbit extends outward as the basal plate. Anterior to this plate and running between the orbit and the olfactory capsule is the elongated palatal fossa (*pl.f.*) previously noted in ventral view.

Perforating the brain case in the orbit are numerous foramina through which nerves or blood vessels course between the brain on the one hand and the structures of the eye and the facial region on the other. Ventrally and a little in front of the middle of the orbit is a large opening, the optic foramen (*f.II*), through which the optic nerve reaches the brain. Above and slightly anterior to the optic is a small trochlear foramen (*f.IV*) through which the fourth cranial nerve passes to the superior oblique muscle of the eye. Behind the optic foramen, and in the lower

posterior angle of the orbit, is the large facial foramen (*f.VII*), through which branches of the seventh or facial nerve pass. Almost in the same foramen but slightly ventralward and forward is a small opening for the entrance of the external carotid artery to the orbital region (*f.e.c.'*). Between the facial and optic foramina is a small perforation for the entrance of an artery, the ramus anastomoticus of Hyrtl (*f.r-a.*). This, in Gegenbaur's ('72) plate 2, figure 1, has been marked incorrectly the 'Querer Basalcanal.' Above the facial is the large orbital fissure (*o.f.*) (trigeminal opening) through which pass the fifth, sixth and the first part of the seventh cranial nerves. Slightly above the middle part of a line connecting the orbital fissure and the optic foramen is the oculomotor (*f.III*) for the exit of the third cranial nerve from the brain to muscles of the eye. Between the orbital fissure and the foramen for the ramus anastomoticus artery is the interorbital canal (*i.o.*) by means of which the orbital sinuses of the two sockets communicate. In the antero-dorsal angle of the socket are two foramina, the larger and upper of which is the ophthalmic (*f.o.VII*) for the superficial branch of the seventh nerve; the smaller and more ventrally placed is for the deep ophthalmicus profundus (*f.o.p.*). In the last mentioned opening is a second smaller foramen for the anterior cerebral vein. This leaves the cranial cavity in the region above the olfactory lobe. Below this, in the anteroventral angle of the socket is the posterior entrance to the orbito-nasal canal (*o-n.*) through which a vein passes from the olfactory region. (For the anterior end of this canal see *o-n.'*, fig. 2.)

A median sagittal section through the cranium (fig. 5) shows the cavity for the brain. Surrounding this are the walls of the brain box through which the foramina lead. Dorsally the cranial roof or tegmen cranii varies considerably in thickness. Posteriorly and above the foramen magnum (*f.m.*) is a thick portion through the occipital crest (*o.cr.*). Anterior to this the wall pits sharply downward forming the parietal fossa. From this fossa the roof again arches upward and then, as the parieto-frontal groove, passes forward to the anterior fontanelle.

From the fontanelle anteriorly the walls are extended by the rostral and basitrabecular cartilages.

Along the ventral margin the floor or basis cranii also shows extremes in thickness. Directly under the anterior fontanelle it is relatively thick. It then becomes thinner and thinner posteriorly until it reaches the foramen for the internal carotid artery (*f.i.c.*). As we have said (p. 450) this foramen divides the basis cranii into two parts, the anterior of which is the embryonic trabecular, and the posterior the parachordal region. The parachordal or the part accompanying the notochord is greatly thickened. It extends to the posterior part of the cranium as a somewhat spool-shaped segment. Inside of this is the cranial notochord (*ch.*), and surrounding it posteriorly are calcified tissues.

Posterior to the end of the cranial notochord but not in the middle line appears the occipital condyle (*o.cd.*). Other structures are seen below the basis cranii and in the background. These in front of the occipital condyles are the post- and pre-hyomandibular processes; under the socket is the down-curving basal plate; and under the anterior fontanelle, the margin of the palatal fossa.

The foramina perforating the cranium are here seen to advantage. The most anterior of these is the large opening through which the olfactory tract passes (*f.I*). Midway between this and the occipital condyle is the optic foramen (*f.II*); above and anteriorly is the anterior cerebral foramen (*f.a.c.*). Almost directly above the optic is the trochlear (*f.IV*). Slightly posterior to the optic are two foramina, the upper for the oculomotor nerve (*f.III*), the lower for the ramus anastomoticus artery (*f.r-a.*). Above the entrance to the internal carotid artery is the inter-orbital canal (*i.o.*). Above the anterior tip of the cranial notochord are two foramina, the upper of which is the large orbital fissure (*o.f.*); the lower of the two is a double foramen, the anterior division of which is for a part of the facial nerve; the posterior gives the acoustic or eighth cranial nerve access to the ear (*f.VIII*). Posterior to this is the smaller foramen for the glossopharyngeal nerve, behind which is the larger

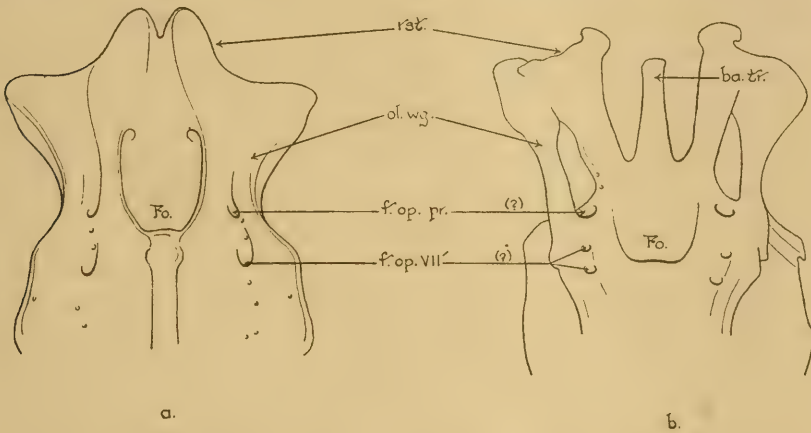
foramen for the vagus (*f.X*). Below the vagus are three smaller foramina (two in fig. 5) through which the spino-occipital nerves of the ventral root type pass. Finally between the posterior end of the cranial notochord and the ventral margin of the occipital crest is the large foramen magnum (*f.m.*).

At the posterior end of the cranium (fig. 4) ventral to the foramen magnum is the cranial notochord, at the sides of which are the occipital condyles (*o.cd.*) by which the spinal column is joined to the cranium. At the side of and slightly above each condyle is the large foramen for the vagus nerve (*f.X*); above this are two smaller foramina through which a spino-muscularis artery (*f.s-m.*) perforates the cartilage without entering the brain case. These, I take it, are the foramina which Haswell ('84, p. 93) describes for *Crossorhinus* as "a pair of small apertures of unknown function." Still further laterally is the opening for the glossopharyngeal or ninth cranial nerve (*f.IX*). At the lower angles of the posterior part of the cranium are the post- and superior articular processes (*po.hm.* and *s.hm.*) by which the hyomandibular suspensorium is fixed to the cranium.

A view of the rostral region (fig. 1 and text fig. A) explains the structures there involved. It is noted that the pointed anterior end bifurcates into dorso-lateral halves which, near the middle line, bend downward and fuse with the median ventral trabecular piece. These, in *Heterodontus francisci*, were they compared with a form in which the rostral cartilages compose a well-marked framework, as for example *Pseudotriacis microdon* (Jaquet '05), would show but slightly their rostral nature. A comparison with another type is instructive in this respect. In *Crossorhinus* (*Orectolobis*) Haswell ('84) figures a cranium (*b* of text fig. A) in which he describes paired pieces (*rst.*) as being prolongations of the ventral floor. From the condition present in the heterodont sharks it would seem not improbable that they are in fact projections from the dorsal and the lateral walls rather than from the floor. If such be true the likeness between *Crossorhinus* and *Heterodontus* in this regard is striking, for a union of the median rostral piece (*rst.*) with the olfactory wing (*ol.wg.*) above and of the olfactory wing and the basitran-

becular cartilage (*ba.tr.*) below would give to *Crossorhinus* a type of rostrum much like that of *Heterodontus*.

b. The *visceral skeleton* is composed of a series of right and left cartilaginous arches which more or less completely surround the buccal cavity and the pharynx. These, in *Heterodontus*, if viewed, say, from the left side, are like those of other pentanchid sharks, seven in number; they may be divided into two groups. The first group comprises the first and second arches,



Text-fig. A. Nasal region of *Heterodontus francisci* and *Crossorhinus barbatus*; *ba.tr.*, basi-trabecular cartilage; *f.op.pr.*, ophthalmic foramen for profundus nerve; *f.op.VII'*, ophthalmic foramen for facial nerve; *Fo.*, anterior fontanelle; *ol.wg.*, olfactory wing; *rst.*, rostral cartilage.

the first of which, the mandibular, is composed of the upper and the lower jaw; the second, the hyoid arch, is similarly made up of two segments. The second group consists of five branchial arches which support the pharynx. In structure the branchial arches are essentially similar to the first two arches excepting that in these there are typically four segments to an arch. These differ among themselves, however, in minor details.

The mandibular, or first arch (fig. 7), has become the most highly specialized of all the visceral arches. Its upper segment, representing the palatal and quadrate regions, is called the

palato-quadrate (*p-q.*); the lower segment is the mandibular or Meckel's cartilage (*md.*). In *Heterodontus* this arch is closely attached to the cranium in the preorbital region by a capsular ligament which keeps the upper and anterior margin (*a.p.*) of the palato-quadrate in the palatal fossa of the cranium. A slip from the capsular ligament (*c.l.*' fig. 7), arising along the ventral margin of the cranium under the fossa (*fig. 2), extends backward and downward to join the quadrate on the ventral part of the transverse median ridge (*tr.m.r.*). Just under the orbit both the upper and the lower segments of the mandibular arch flare outward in *Heterodontus francisci* so that the distance to the spiracular cartilage or to Huxley's so-called otic process is, I take it, greater than that described by Huxley for *Heterodontus philippi*. Posteriorly, the arch has no direct attachment to the cranium but is held in position by ligaments soon to be described.

As a cartilage the palato-quadrate (*p-q.*, figs. 6 and 7) is longer than the mandible. Its upper margin is irregular, due principally, to a dorsal indenture in the anterior third. The anterior wall of this indenture comes in contact with the ethmoidal region, while the posterior wall of the indenture, as seen in figure 7 (*a.p.*) serves as a process of the palato-quadrate which fits into the palatal fossa of the cranium. Medially from this articular surface a sharp ridge (*tr.m.r.*) runs, to the lower part of which is attached the slip from the capsular ligament above described. Externally, at the beginning of the posterior third of the quadrate there is a strong lateral transverse ridge which passes almost across the cartilage (*tr.l.r.*, fig. 6). To this ridge are attached tendinous fibers of the adductor mandibularis muscle. Posteriorly and dorsally the palato-quadrate is provided with a lateral flattened enlargement, the hyal process (*hl.p.*), also for muscular attachment. The hyal process is continuous with a similar process on the mandible.

The mandible (*md.*, figs. 6 and 7) is an unusually heavy cartilage. The angular part of this is high, considerably elevating the quadrato-mandibular joint. If seen from below, the mandible would appear as a strongly crescentic cartilage, the poste-

rior tip of which extends considerably laterad of the anterior. Inside of and below the teeth, there is a long ridge (*md.r.*, fig. 7) from which a tendinous bridge passes to a similar ridge on the other side; to the lower sides of this ridge the strong coraco-mandibularis muscle is attached; near the quadrato-mandibularis joint and mediad there is present a prominent mandibular knob (*kb.*) against which the second arch abuts.

The joint between the palato-quadrate and the mandible, like that in *Chlamydoselachus* (Goodey '10, pp. 544-545) and *Heptanchus* (Gadow '88, pp. 452-453) forms a double ball and socket. The anterior articulation is formed by a ball of the mandible fitting into a socket of the quadrate. The posterior articulation consists of a large socket in the outer angular part of the mandible, into which a ball from the hyal process of the quadrate fits. Between the two articulations in *Heterodontus* is a space, somewhat like that described by Gadow ('88) for *Heptanchus*.

A description of the articulations of the first arch, further than the attachment of the palato-quadrate to the cranium as above described (p. 456), may be deferred until a study is made of the second or hyoid arch.

The hyoid arch (fig. 6) as we have seen, is also composed of two segments. The upper division, the epihyoid, becomes in *Heterodontus* an important suspensorium for the mandibular or first arch; it is called the hyomandibula (*hm.*). The lower segment of the arch is the ceratohyoid or hyoid proper (*c-h.*). Connecting the two ceratohyoids of opposite sides is a median unpaired piece, the basihyal cartilage (*b.h.*, fig. 11).

Both of the segments of the hyoid arch are heavy cartilages. The hyomandibula is thickened both proximally, where it fits into the deep fossa under the auditory capsule, and distally, where it joins the ceratohyoid and touches the mandible near the quadrato-mandibular joint. The ceratohyoid is considerably longer than the hyomandibular segment, and extends forward and inward to meet the basihyal.

Articulations of the hyoid arch. The hyomandibula is bound by a strong capsular ligament to the hyomandibular fossa in the

cranium. This, in figure 6, has been removed so as to show the proximal end of the hyomandibula. The superior post-spiracular ligament (*s.p-s.l.*) of Ridewood '96; (see also W. K. Parker '79), arising in the postero-ventral angle of the socket and anterior to the auditory capsule, attaches itself to the distal third of the hyomandibula. Further, the hyomandibula is bound to the ceratohyoid by a hyomandibulo-hyoid ligament (*l.hm-h*, fig. 6) which arises on the side of the distal end of the hyomandibula and passes over to the anterior and inner face of the ceratohyal segment, extending thence to its distal third.

A series of ligaments may next be described which are effective in swinging the first or mandibular arch, all but one of which connect this arch directly to the second. That one, however, indirectly and in part, attaches the first arch to the cranium. Those binding the first arch to the second directly and appearing externally are three in number. The first of these is a dorsal ligament (*l.hm-q.*, fig. 6) which passes from the upper part of the hyal surface of the quadrate posteriorly to the medial and anterior part of the hyomandibula. This ligament is doubtless that part of the superior post-spiracular ligament which Ridewood ('96, p. 427) described for *Scyllium* as attaching on the quadrate. In *Heterodontus francisci*, however, its attachment is on the hyomandibula, few of its fibers being continuous with the superior post-spiracular ligament. I have therefore called it by a separate name, the ligamentum hyomandibulo-quadratum (*l.hm-q.*).

At the joint there is a complex median ligament (*l.m.*, figs. 6 and 7) which passes from the inner side of the quadrato-mandibular joint externally, principally, to the cerato-hyoid cartilage. The quadrate part of this ligament (*l.m.*, fig. 7), however, arising under the large ligament which joins the mandible to the quadrate (*l.q-m.i.*), runs upward and posteriorly to attach to the hyomandibula, mediad of and slightly distal to the attachment of the ligamentum hyomandibulo-quadratum. All of those fibers of the median ligament which arise from the joint and from the mandible (*l.m.*, fig. 7) are attached to the ceratohyoid.

From the ventral angle of the mandible a third ligament, the ligamentum hyoideo-mandibulare (*l.h-m.*, fig. 6) (the ligamentum hyoideo-mandibulare externum of Goodey '10) extends posteriorly to be attached to the inner margin of the ceratohyoid segment. Near the mandibular attachment this ligament is perforated by an artery.

A most complex suspension is made by a ligament which passes from the medial side of the mandible to the ventral side of the cranium. For want of a better name I shall call it the 'ligamentum complexum' (*l.cp.*). Only a part of its course can be shown in figures 6 and 7. This arises as a double band (*l.cp.*, fig. 7), ventral to the mandibular knob, and passes outward over the ceratohyoid to which some of its deeper fibers are attached (*l.cp.*, fig. 6); it then gives a bundle of fibers to the hyomandibula and, with fibers from the hyoid, runs upward and mediad of the hyomandibula to be attached ventrally to the base of the cranium slightly anterior to the external carotid foramen (see fig. 2). It seems probable that the upper part of this ligament at least is comparable to the inferior post-spiracular ligament of Ridewood ('96).

It is thus seen that, excepting the suspension rendered by this complex ligament just described and by a few fibers from the ligamentum hyomandibulo-quadratum, the first arch in the posterior region is suspended *entirely* by the second. The attachment of the first by the second is so complex that it would be hard to agree with Huxley ('76, p. 43) that "The 'epibranchial' (hyomandibula) of the hyoidean arch of Cestracion (Heterodontus) is just *beginning* to take on a new function, that of suspending the palato-quadrato cartilage and mandible to the skull."

It may be added that the union of the quadrate and mandibular cartilages is made principally by a large ligament, the ligamentum quadrato-mandibulare internum (*l.q-m.i.*, fig. 7) already mentioned. This attaches to the upper internal border of the quadrate above and extends along the posterior border of the transverse median ridge of the palato-quadrato (*tr.m.r.*) and over the anterior articulation of the quadrato-mandibular

joint to be attached to the lower ventral margin of the mandible. Further, it may be said that a slip from this ligament, the ligamentum hyomandibulo-mandibulare (*lhm-m.*), passes upward to be attached on the hyomandibula, just proximal to the quadrate slip of the median ligament and directly under the attachment of the hyomandibulo-quadratum. It thus results that a puncture through the fibers of the superior post-spiracular ligament and through the attaching fibers of the ligamentum hyomandibulo-quadratum would perforate the attaching fibers of this ligament.

Finally other ligaments may here be mentioned. Strong fibrous bands run lengthwise of the lateral or concave surface of the mandible and the palato-quadrate to the quadrato-mandibular joint. The one on the mandible sends a slip upward to attach on the quadrate just mediad of the first (anterior) articulation. A similar slip from the quadrate attaches on the mandible just mediad of the second (posterior) articulation. These two attaching ligaments form a curious type of scissor ligament.

The cartilaginous gill-rays. There are present on the hyomandibula (epihyal) and on the ceratohyal segments of the second arch, cartilaginous rays which project outward and backward as supports for the gill septa. These show considerable variation in different specimens. In one of the large males the first six of these on the hyomandibula fuse at their proximal ends into two masses. The sixth is followed by nine single rays which meet and fuse at their proximal ends into a half arch. Eight single rays of the ceratohyal, similarly fusing at their base, complete the arch. This arch then encircles the articulation between the two segments. Following these upper ceratohyal rays there are six pairs of rays fused at the bases into three pieces, and following these there are two or three stout rays.

The first branchial arch (fig. 10) consists of four segments, three of which are shown in figure 10. These, counted from the dorsal to the ventral side, are: (1) the pharyngobranchial (*p-b.*), (2) the epibranchial (*e-b.*), (3) the ceratobranchial (*c-b.*), and (4) the hypobranchial (*h-b.*, see fig. 11). From below, the

arch slants obliquely backward so that the pharyngobranchial is considerably behind the outer segments of the arch.

The upper or pharyngobranchial segment is a triangular-shaped cartilage, the apex of which points ventro-laterally, and the broad base of which forms its dorso-median margin. It is not bound by pronounced ligament to either the pharyngobranchial of the opposite side or to the spinal column, but it is held in place dorsally by connective tissue. A ligament is attached to the neck of the pharyngobranchial just above its union with the epibranchial segment. To a further consideration of this ligament we shall return. Here it may be said simply that it passes posteriorly to the head of the following epibranchial segment.

The epi- and ceratobranchials are stout cartilages, the latter being considerably longer than the former. Near their joint both cartilages are hollowed out (not seen in fig. 10) so as to increase the angle between the two. The joint between them is simple, the articulating surfaces being held together by a connective tissue capsule.

The epi- and ceratobranchial segments are of great importance to the area since they alone possess cartilaginous branchial rays for the support of the gill tissues (*b.r.*, fig. 10). On the first arch fourteen such rays are usually present, five on the epi- and nine on the ceratobranchial segment; the first on the ceratobranchial is in all cases the longest of the series.

The hypobranchial segment of the first arch (*h-b.1*, fig. 11) is much smaller than any of the other segments. It is not connected with the hypobranchial of the other side or with a median basibranchial cartilage, but remains as a rudimentary cartilage connecting the ventral ends of the first ceratobranchial with the cerato- and basihyal cartilages.

Dorsally the segments of the second, third and fourth arches are essentially like the first, except that the pharyngobranchial segment of the fourth has fused with that of the fifth arch (fig. 14). Ventrally, these arches differ from the first principally in that their hypobranchial segments are well developed (*h-b.2-4*, fig. 11). The hypobranchial segment in these is so arranged as to

be attached by pads of tissue to the end of its own ceratobranchial and also to that of the ceratobranchial just in front. These hypobranchials then run posteriorly and medially to join unpaired cartilages soon to be described.

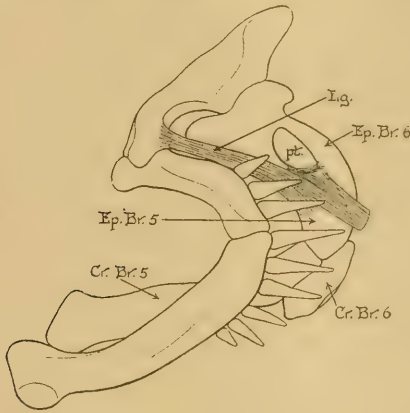
The fifth branchial arch is greatly modified (fig. 14). Its pharyngobranchial segment has been so distorted as almost completely to change the appearance of the upper part of the arch. Its epi- and ceratobranchial segments (*e.b.5* and *c.b.5*) are stout cartilages, devoid of branchial rays. The hypobranchial segment is absent, and its ceratobranchial is attached directly to a median unpaired cartilage—the basibranchial (fig. 11).

The median unpaired pieces and their relation to the segments of the arches are shown in figure 11. The most anterior of these is the basihyal cartilage, to which reference above has already been made and which may be described as a more or less star-shaped cartilage joining the right and left halves of the ceratohyoids in the mid-ventral line. It has an anterior triangular glossal projection (*g.p.*) which bends upward in the floor of the mouth to form a support for the so-called tongue.

A first basibranchial cartilage has been mentioned by White ('92, p. 299) as characteristic of *Heterodontus* (Cestracion). (See also Gegenbaur '72, pl. 19, fig. 3). This, according to Gegenbaur, is located in *H. philippi* as a free nodule of cartilage in the middle line, posterior to the basihyal cartilage. For the same form Karl Fürbringer, ('03) describes paired basal cartilages. In *Heterodontus francisci* I have not found either the azygos cartilage of Gegenbaur or the paired cartilages described by Fürbringer. I have found, however, cartilages in this region which I have described as extra-hyoid cartilages. These are identical in shape and direction with those given by Fürbringer; but I am convinced that they are not first hypobranchial cartilages since they lie *superficial* to the afferent artery. The second basibranchial forms a large median piece (*b.b.*) to which the hypobranchials of the second branchial arch are united. Posteriorly this median piece joins an enlarged backwardly-directed, arrow-shaped piece (*m.p.*) to which the third and fourth pairs of

hypobranchials and the fifth ceratobranchials are attached. This piece bears a posterior segment.

The discovery of a vestigial sixth branchial arch in the Heterodontidae (Hawkes '05) has been of considerable interest. This arch was found in the young both of *Heterodontus francisci* (*Gyropleurodus francisci*) and of *H. philippi*. Since this discovery, however, an additional rudimentary arch has been described in several other elasmobranchs.



Text-fig. B Rudimentary sixth branchial arch of *Heterodontus francisci*; *Cr.Br. 5.*, fifth ceratobranchial cartilage; *Cr.Br. 6.*, sixth or rudimentary ceratobranchial cartilage; *Ep.Br. 5.*, fifth epibranchial cartilage; *Ep.Br. 6.*, sixth or rudimentary epibranchial cartilage; *Lg.*, ligament passing under pit; *pit.*, pit in neck of pharyngobranchial cartilage.

The rudiments of the arch found by Hawkes in *Heterodontus francisci* consist of a pair of pieces located back of the fifth arch. The upper piece is attached by a ligament to the epibranchial segment of the fifth arch and is joined below to the second rudimentary segment. These pieces are interpreted by Hawkes either as the cerato- and hypobranchial, or as the epi- and ceratobranchial segments which have become closely joined to the fifth arch.

In the adult specimen these cartilages (*ep.br. 6* and *cr.br. 6*, text-fig. B, and fig. 14) are much like those described for the

young. The upper segment (*ep.br.6*) is a slender cartilage, (loosely) attached dorsally to the fifth pharyngobranchial, but ventrally, as is seen from the figure, it is fused solidly to the fifth epibranchial segment. If viewed from the median side, however, it is seen to be continuous with the lower segment.

I have already described for the first branchial arch a ligament which extends from the neck of the pharyngobranchial backward and downward to be attached to the epibranchial following. To this I shall now return with a hope that it may be of service in interpreting the dorsal piece in question. It so happens that this ligament forms the floor of a pit, the anterior wall and the roof of which are formed by the neck of the first pharyngobranchial and the posterior wall by the upper part of the following *epibranchial segment*.² Similar pits follow the second, third and fourth (*lg.*, text fig. B) arches. Now, following the fifth pharyngobranchial there is also a pit (*pt.*, text-fig. B) which, in its surroundings, is identical with the first. From the neck of the fifth pharyngobranchial the ligament passes under the pit, giving some of its fibers to the posterior wall; other fibers from the fifth pit continue across with the ligament from the fourth pit to join the pectoral girdle. The piece forming the posterior wall (*ep.br.6*) I therefore interpret as the sixth epibranchial segment.

The lower or second segment, the ceratobranchial, is displaced forward so as to lie laterad of the joint of the fifth arch. This displacement in *Heterodontus* is due to the enlargement and crowding forward of the massive pectoral girdle.

There is present in the anterior wall of the spiracle a thin spiracular cartilage (*sp.c.*, pl. 3, and fig. 12) which, like branchial rays, supports a septum for gill filaments. This cartilage is (generally) interpreted as a fused series of cartilaginous rays which originally belonged to the palato-quadrate segment. Because of the outward flaring of the quadrate the spiracular cartilage comes to be widely separated from the quadrate.

²This ligament probably represents the median interarcuales muscles.

Extra-visceral cartilages. The visceral arches are provided with superficial pieces, the extra-visceral cartilages. These for convenience may be separated into the labial cartilages, the extra-hyoid and the extra-branchial cartilages. The labials are located at the sides of the mouth and consist of three pieces of cartilage on each side, two dorsal and one ventral (*d.l.1-2* and *v.l.*, fig. 6). The posterior dorsal labial is about twice the length of the anterior dorsal labial cartilage; it articulates with the ventral labial at its distal end so that the two serve to reduce the gape of the mouth.

An extra-hyoid cartilage, so far as I have been able to make out, is lacking dorsally, and the one which appears ventrally is small. This, in the adult, is generally a nodule of cartilage less than half the length of the one shown in figure 9. In all cases the extra-hyoid cartilage was located where the termini of the ventral aorta bifurcate to form the first and second afferent arteries, the body of it lying superficial to the base of the second afferent. In no case did I find it further out over the first gill pocket as is shown for *Heterodontus philippi* (Max Fürbringer '97, pl. 6, fig. 5). In one of the cases examined the extra-hyoid on the right side was elongated as is shown in figure 9, while on the left it was a nodule very much like the enlarged end of the cartilage seen in figure 9. Usually both of the cartilages were almost identical in shape with that figured by Gegenbaur and Karl Fürbringer as the first basibranchial. I am at a loss to know whether what Gegenbaur described as a single piece and Fürbringer described as paired cartilages are not in fact what I have regarded as the extra-hyoids. If these be the cartilages described by them I am convinced that they are not basibranchials since they lie entirely superficial to the second afferent artery.

Extra-branchial cartilages are located over all of the (internal) branchial arches in *Heterodontus* excepting the fifth. The extra-branchials of the first branchial arch are usually large and like succeeding arches overlap terminally (*ex.b.*, fig. 10). The first three cartilages are hook-shaped at their attached ends, the dorsal ones only slightly and the ventral pieces in a very pro-

nounced fashion. In both dorsals and ventrals the body tapers toward the free end. The fourth extra branchial (not added in fig. 14) is smaller and simpler in form both above and below, than the preceding.

The four dorsal extra-branchial segments are united in such a way as to make the upper attachment in a continuous line above the gill pockets. Each of these cartilages curves around the tip of the branchial rays at the margin of a gill septum. The attachment of the lower or ventral extra branchials is less concentrated. The first three of these are joined to the connective tissue ventrally at the base of their respective gill-pockets, but the fourth is shorter and tends to migrate upward so as to rise from the side of its ceratobranchial.

2. *The spinal column*

The spinal column in *Heterodontus*, although somewhat variable in the number of its segments, consists of about one hundred and ten clearly marked vertebrae. Of these the first thirty-one have ribs growing from their basiventrals (*b.v.*) or transverse processes; the five or six following these have their basiventrals bent downward, and the sixth or seventh (thirty-seventh or thirty-eighth of the column) usually has them meeting below to form the first hemal arch. Thirty-four similar arches follow back from the first haemal arch to the beginning of the ventral rays of the tail (on the seventy-first vertebra). There are next thirty-nine or forty caudal vertebrae, behind which in the adult is a mass but slightly differentiated, tapering gradually to a point (see also T. J. Parker '87, p. 31, pl. 8, fig. 28).

The first vertebra in the column (fig. 15) is obscured by an incomplete anterior segment having a short centrum and bearing enlarged transverse processes for articulation with the occipital condyles of the cranium. The second complete vertebra (*vt.*², fig. 15), appearing behind the incomplete segment, may be described as provided with a strong centrum upon which rests a basidorsal (basal) piece (*b.d.*²), above and posterior to which is a large interdorsal (intercalary) plate (*i.d.*²). Both of these plates are perforated, the former giving passage to the ventral root

nerve (*f.v.*), the latter to the dorsal root (*f.d.*) of the first spinal nerve. Capping the column between the first and second interdorsal plates on each side is a smaller plate—the suprabasidorsal (*s.b.d.*) or neural spine. The centrum itself has extending from its side a basiventral (*b.v.*) from which the rib (*r.*) projects.

In an end view of the fifth vertebra the structures there concerned are seen to advantage (fig. 18). Resting upon the centrum is the neural arch (*n.a.*), the sides and the top of which are made up entirely of the cut edge of the interdorsals (*i.d.*), but the basidorsal plates (*b.d.*) of the vertebra appear in this end view. At the ventro-lateral angles of the centrum are the ribs (*r.*) which stand out almost at right angles.

A similar view taken of a vertebra toward the tail region, say the fifty-third, in addition to a neural arch above, shows a haemal arch below the centrum (*h.a.*, fig. 21). The neural arch in this case shows the interdorsal plate with a basidorsal (*b.d.*) seen in end view. The haemal arch is formed by the downward bending of the basiventrals and by the adjoined haemal spine; the basiventrals make up the larger part of the wall and all of the floor of the canal. Contiguous to the centrum, however, are seen the interventral plates of the vertebra. In such a view it will be observed that the haemal canal is divided by a partition into a dorsal and a ventral part, the dorsal being for the caudal aorta and the ventral for the caudal vein.

If a series of transverse sections be taken through the two above vertebrae, the ends of which are figured (figs. 18 and 21), the composition of the arches as well as the finer structure of the vertebrae may be made out. The neural arch in the second section (fig. 19) shows a great extent of the basidorsal cut and a decrease in the amount of the interdorsal. In the third section (fig. 20) the suprabasidorsal or neural spine (*s.b.d.*) also appears, so that all three of the parts making up the arch are seen here. In the caudal vertebrae the neural arch is similar to that just described. In figure 22 a bit of the suprabasidorsal is cut, and in both figures 22 and 23 the haemal arch shows the basiventral, that part of the interventral which appears in end view (fig. 21) not being touched.

A study of the finer structure of these vertebrae is of interest. At the end of the vertebra the calcified tissue appears as a whitish ring like the walls of a funnel (*cl.r.*, figs. 18 and 21). In this funnel is to be found primitive gelatinous notochordal substance which at the ends also forms an intervertebral pad. As the sections pass farther toward the middle of the centrum this calcified ring becomes smaller and smaller, and there pass off from it numerous radiating calcified plates (*cl.p.*). As the middle of the centrum is reached (figs. 20 and 23) the apex of the funnel appears as a tiny circle of calcified tissue containing the constricted part of the notochord (*ch.*). The calcified ring (*cl.r.*) may here be likened to a hub from which the calcified plates radiate like the cogs of a wheel. It will be noticed that the calcified ring diminishes in thickness as well as in size and that the cogs decrease in number the nearer we approach the middle of the centrum (compare figs. 19–20, also 22–23), showing that some of these do not extend the entire length of the centrum. It is also to be seen that the cogs are not so numerous in the region of the fifty-third vertebra as in the region of the fifth.

Other calcifications of less importance also appear in the vertebrae. These are confined principally to the lining of the neural canal, to the outside circle around the body of the centrum (not shown in the figures), and to the roof and sides of the haemal canal.

Considerable interest attaches to the region of the spinal column between rib-bearing and the haemal arch segments. This area, which may be designated as a transition between the body and the caudal regions, may be represented by a section of the column extending from the thirtieth vertebra—the last but one to bear ribs—to the thirty-eighth, usually the second to form a complete haemal arch (fig. 16). In such an area there is a sudden change in length of centrum, the centra of the non-rib-bearing vertebrae being markedly shorter than those of a rib-bearing nature. This change begins usually with the thirty-second vertebra but in one case I found that the thirty-second was still fused with the succeeding vertebra dorsally, although the two were separate ventrally. On the opposite side, however,

the two plates were fully separate both above and below. In *Heterodontus francisci* only six or seven vertebrae form the transition between rib-bearing and haemal vertebrae (see also Gegenbaur '67, pl. 9, fig. 19, and Šecérov '11, pl. 1, fig. 5). Upon these centra and those following, the plates of the neural arches are also modified, being so arranged that to each myomere two basidorsal and two interdorsal plates occur. Such vertebrae are called diplospondylous.

In the transitional vertebrae alternate basidorsals, which are odd in number, are perforated by the ventral roots of the spinal nerve (*f.v.*), and alternate interdorsals are usually separated by the foramina of the dorsal nerves (*f.d.*). On the thirty-seventh vertebra (fig. 16) the foramen for the dorsal root, however, migrates forward so that it perforates the interdorsal (intercalary) plate. In some other cases the dorsal root foramina pass between the interdorsal and the basidorsal, producing a more or less pronounced niche in the anterior part of the basidorsal plate. In this transitional area, the suprabasidorsal (*s.b.d.*) above imperforate basidorsals is single where the foramen completely separates these. Above each perforate basidorsal it is usually doubled. These occur in regular fashion, capping the interspaces between basidorsals and the interdorsals so that in the case of figure 16 they comprise three (pairs of) nodules to each myomere.

It will be observed, further, that haemal arches corresponding to the imperforate and perforate segments may be determined by their shape without reference to the basidorsals in question. This will be made especially clear by reference to the fortieth and forty-first basiventrals. The ventral termini of these point in opposite directions, the fortieth pointing anteriorly, and hence corresponding to the imperforate basidorsal, and the forty-first posteriorly, and hence belonging to the same segment as the perforate basidorsal. In a later study of the caudal region we shall have reason to refer to the foramen formed between the haemal arch of the imperforate segment (anterior) and the haemal arch of the perforate segment (posterior). It is through this that the main segmental artery leaves the caudal aorta.

The question has been raised: Do the diplospondylous vertebrae return to the monospondylous type as the tip of the tail is approached. This is stated to occur in *Acanthias* by Ridewood '99), who says, "The change from diplospondylous to the monospondylous condition occurs at about the twenty-fourth centrum from the end." In *Heterodontus francisci* such is not the case (fig. 17). It is difficult, as Ridewood says, to delimit a myomere in the posterior region because of the thinness of the muscle, yet in an injected specimen the segmental arteries arising from the caudal aorta are clearly definitive of boundaries, only two to a myomere, one in front of it, the other bounding it posteriorly. Since two vertebrae occur between each two arteries, at least as far back as the ninety-eighth segment, the diplospondylous nature is retained. Some of the segments posterior to this retain their regularity although in these the arteries themselves are not sufficiently regular to be definitive.

II. THE APPENDICULAR SKELETON

The part of the skeleton known as appendicular consists of pectoral and pelvic girdles and the frame-work for the fins attached thereto.

1. *The skeleton of the fin girdles*

a. *The pectoral girdle* in *Heterodontus* (fig. 8) is a strong arch, open dorsally, to which the frame-work of the pectoral fin is attached. It is composed of a right and a left cartilaginous part solidly fused in the middle line below. The part of the girdle which extends dorsal to the attachment of the fin is the scapular portion (*sc.*). That part which meets a similar part from the opposite side below is the coracoid portion (*co.*). At the middle of the postero-lateral portion of each half of the girdle is a projection or articular surface (*a.pt.*) which fits into the fossa of the pectoral fin skeleton. In front of this articular surface is a strong antero-ventral projection (*a.pr.*), like that in *Crossorhinus* (see Haswell '84), to which is attached strong musculature. A foramen through which nerves and blood vessels pass

perforates the girdle between these two projections. Above the articular process (*a.pt.*) and on the scapula is the postscapular projection to which the heavy lateral musculature attaches. Between the anterior projection and the mid-line there is a deep concavity on the coracoid from which the *arcuales communis* muscles arise.

b. The pelvic girdle (fig. 13) consists of a flattened bar—slightly cupped up in the middle and expanded at the ends. The right and left halves of this are firmly fused together. Three foramina perforate the pelvic girdle near each end (*f.pl.*); through the median one the iliac artery passes, and through the other two, nerves. At the termini of the girdle are the articular processes for the right and left fins. Each articular surface consists of two protuberances (*a.pl.*) which fit into depressions of the fin skeleton proper.

2. The skeleton of the fins

a. The paired fins. 1. The skeleton of the pectorals (fig. 26) is made up of a group of large basal cartilages from which radiate numerous rows of radialis. The basal cartilages in the pectoral of *Heterodontus francisci* are unlike those previously described for the adult of *Heterodontus philippi*. In the former there are three pieces, pro- meso- and metapterygium, the first (the propterygium) being absent in the adult of the latter species. (Mivart '79, p. 449; Huxley '76, p. 50; and Gegenbaur '65, II, pl. 9, fig. 3; see also Howes '87, pl. 3.)

This propterygial basal (*pr-p.*) in *Heterodontus francisci* is a clearly marked cartilage, quadrilateral in shape and somewhat elongated. It is followed by a series of four radialis, the first of which is large and plate-like. Contiguous to this plate-like radial is a hexagonal plate, a part of which evidently belongs to the first radial of the mesopterygium.

The mesopterygium (*ms-p.*) is a stout cartilage, from the enlarged distal end of which five rows of rounder radialis radiate. The most proximal segment of the first row of radialis joins distally the hexagonal plate of the propterygium just described.

In the right fin of my dissection this plate was separated into two parts so as to be included in the first line of the mesopterygial radials. The second row of the mesopterygium consists of six segments. The first segment of the third row of radials is followed directly by a similar more flattened segment which, in turn, abuts against a double row, the anterior of which is composed of four or five flattened plates, the posterior row having but four. The proximal segment in the fourth line of mesopterygial radials, like the third, is followed by a slightly shorter segment which abuts distally against two rows, the posterior of which consists of four radials. The anterior plates have just been described. The proximal segment of the fifth row of radials is the longest of the series. It is continued distally by a pentagonal piece which fits against a double row of radials, the anterior proximal line of which was previously described, and the posterior row has but three segments capped distally by small cartilages.

The *metapterygium* (*mt.p.*) is a spatulate cartilage, the wider part of which points distally. From this ten or eleven rows of radialia diverge. The second two segments in the first two rows of these abut against double rows of radialia, each row usually consisting of two segments capped by smaller pieces; the third, fourth and fifth are similar except that the double rows are usually uncapped. The sixth to the eighth rows are of two segments each, between which terminally single terminal cartilages abut. The proximal segments of the ninth and tenth radials of the metapterygium are followed by segments equal in length to themselves. Each one of these segments is capped by a single piece. In an older specimen the ninth and tenth radials were fused proximally and cleft distally. The eleventh radial gives evidence of being a fusion of two. The accessory cartilages accompany it; one appears at its apex, and another along its edge.

2. The frame-work of the pelvic fin (fig. 29) in the female consists of a long posteriorly projecting basal cartilage, the basipterygium, to which radialia are joined. The basipterygium (*ba.p.*) in *Heterodontus* is separated into five sections, the proxi-

mal of which is long, the distal represented by a tiny cartilaginous tip. Anteriorly a much enlarged fused first radial strikes the basal plate almost at right angles. In the end of this, as well as in the end of the basal piece, is a fossa, by means of which articulation with the pelvic girdle is effected. From this first radial cartilage three rows of radialis project. In the first are one or two small cartilages; in the second, two or three; and in the third there are four cartilages similar to the segments in the first radialis of the basal piece. From the basipterygium thirteen rows of radialis are given off. These are terminated by smaller radial cartilages in the more anterior rows but in the three posterior rows each one consists of two segments.

3. The skeleton of the pelvic fin of the male is, with slight modifications, like that of the female. It consists of the basipterygium and its radials. In the male there are fourteen radials which meet the basipterygium at an angle of forty-five degrees. The most anterior of the radials represents the fusion of three cartilages which, like those following, are segmented into two or three pieces. The most posterior radials, unlike those in the female, are unsegmented. The inner lobe of the pelvic fin in the male (fig. 27) is modified as a framework for the claspers or copulatory organ.

The basipterygium in the male is continued by the basal piece (*ba.*) to which it is connected by two short segments (b^{1-2}). At the angle between the basipterygium and the basal piece there arises from the former the so-called 'beta' (β) cartilage. The basal piece is generally round, but terminally it is flattened and possesses a groove which passes obliquely across to the dorsal side. Several cartilaginous pieces appear near the terminal part of the groove. These are two dorsal terminals (*d.tr.1-2*), a dorsal marginal (*d.mg.*), and one ventral terminal cartilage (*v.tr.*). Proximal to the ventral terminal there is an accessory terminal (*tr.3*).

b. The skeleton of the unpaired fins. 1. First dorsal fin (fig. 24). Extending from the vertebral column one-half the length of the long anterior fin-spine, or to the point externally where the spine emerges from the skin, is a thin basal cartilage, the base of

which extends over two and one-half vertebrae ($17-18\frac{1}{2}$) in the female; (15-18) in the male. From the top of this plate arise two other cartilages, the anterior of which is twice the width of the posterior. From the former there appear four rows of radiating pieces, each row of which contains three cartilages. Capping the second and third rows is an extra cartilage and over the fourth is a similar cap of a double piece.

2. Second dorsal fin (fig. 25). The second dorsal fin, like the first, is provided with a basal piece (*b.c.*) one-half the height of the spine; it extends over three (46-48) diplospondylous vertebrae in the female; (44-48) in male. From it arise three radials which point in a more posterior direction. The most anterior of these is the smallest. Passing from it is a double row of two segments capped with a broader piece. The second radial, though a single piece proximally, is bifid distally. From the distal end arise two rows of three cartilages each. The third radial is a truncate cone which rests on its apex. Upon its base it supports in an irregular fashion six cartilaginous pieces.

3. The caudal fin (fig. 17). The ventral rays of the caudal fin or tail, as we have seen are an integral part of the axial skeleton, being the prolonged haemal spines. These consist of a series of forty rays, each of which corresponds to a vertebra. Beyond the tip of these, in the adult is an undifferentiated (fused) mass. In this region interdorsal pieces are present as far back as the ninety-fourth to the ninety-seventh vertebrae, while interventral pieces extend back to the ninety-ninth vertebra. The dorsal lobe of the fin is supported by rays which unlike those supporting the ventral lobe, are not equal in number to the centra, forty-six being present in one specimen examined. The first of the dorsal rays is small and the second arises as a broad clear piece of cartilage over the seventieth or seventy-first vertebra. Back of this the rays are numerous and are more or less regular to the ninety-fifth vertebra. At the tip of the tail they fuse into a common mass (fig. 17).

In the caudal region alternate basidorsals are perforate back to the ninety-fifth vertebra, with the exception of the eighty-ninth and ninety-third. The eighty-ninth although perforate

on the right side was imperforate on the left. No suprabasidorsals are found just anterior to the dorsal rays, and the interdorsals usually cease at about the ninety-fifth, although they may sometimes extend a few segments further back. It is difficult to make out the exact perforations in the plates posterior to the eightieth vertebra, for they may cut the edges of the plate and hence the nerve may emerge between the plates.

4. The anal fin. The base of the anal fin in the specimen before me abuts against the fifty-eighth centrum of the spinal column. But this position varies between the fifty-eighth and sixty-third vertebrae. From the basal piece, which is remarkably like that of the dorsals, proceed four rows of radials, the first composed of two segments; the second has two rows arising from it, in the first of which are five cartilages, in the second four; the third radial is elongate and is continued by three successively shorter pieces; and the fourth, more elongate still, is followed by a broader radial. Upon this are three rounded pieces, over the first and a part of the second of which is a broader cap.

Berkeley, California
March, 1915

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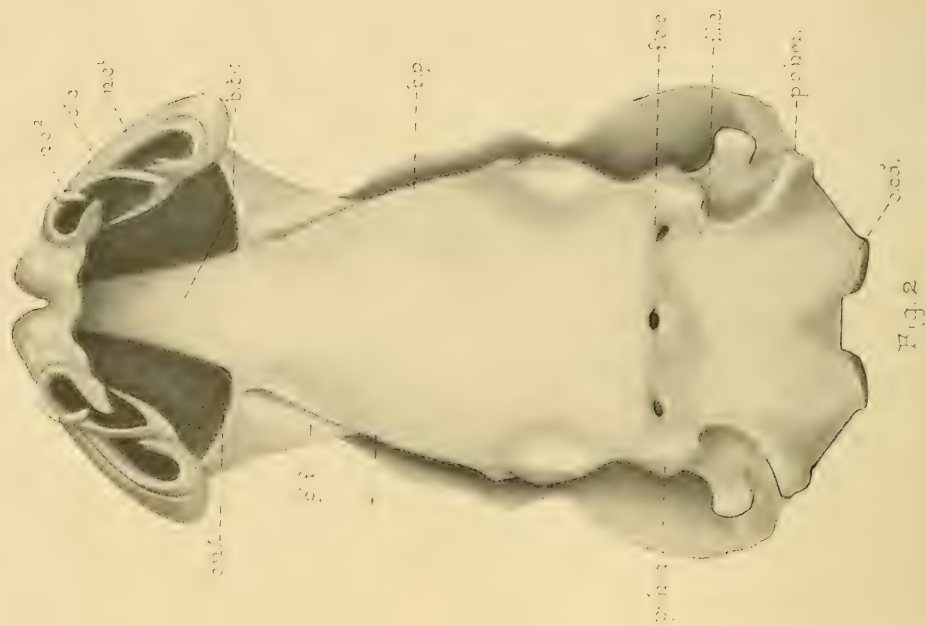
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PLATES

PLATE I

Fig. 1



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PLATE 1

EXPLANATION OF FIGURES

Figures of *Heterodontus francisci* (natural size)

1 A dorsal view of the cranium.

2 A ventral view of the cranium.

a.o.s., anterior oblique semicircular canal

b.p., basal plate

b.tr., basitrabecular cartilage

F., anterior fontanelle

f.e.c., external carotid foramen

f.i.c., internal carotid foramen

f.m., foramen magnum

f.o.p', foramen for the ophthalmicus profundus nerve (exit from the oph. prof. canal)

f.o.VII', ophthalmic foramen for the VII nerve (exit from the VII canal)

n.c. 1-2, first and second nasal cartilages

o.cd., occipital condyle

o.cr., occipital crest

o-n', orbito-nasal canal (entrance from olfactory capsule)

ol.c., olfactory capsule

p.f., parietal fossa

p-f.g., parieto-frontal groove

pl.f., palatal fossa

po.hm., post hyomandibular process

pr.hm., prehyomandibular process

***, attachment of capsular slip

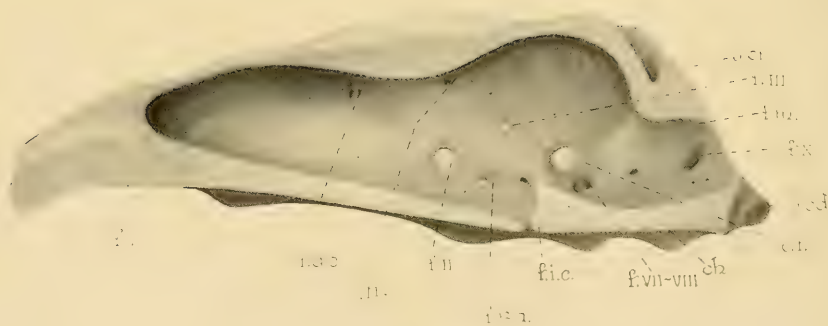
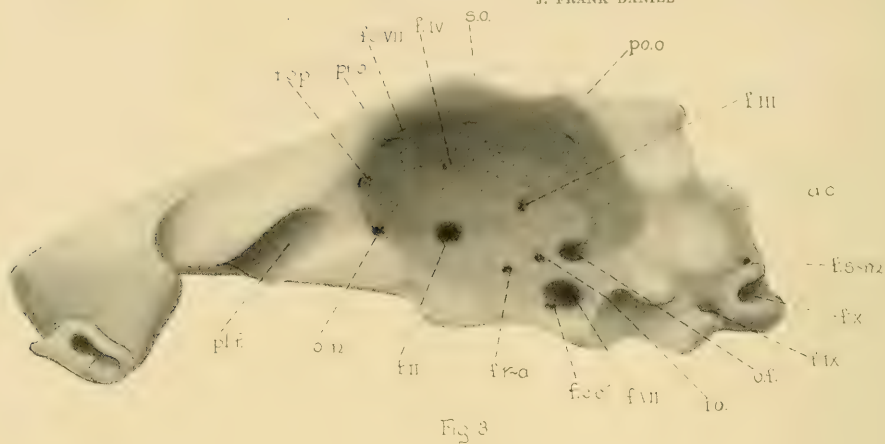


PLATE 2

EXPLANATION OF FIGURES

All figures of *Heterodontus francisci* (natural size).

3 A side view of the cranium.

4 Posterior view of the cranium.

5 A median sagittal section through the cranium, seen from the inside. The nasal capsule has been removed.

a.c., auditory capsule

ch., notochord

f.a.c., foramen of anterior cerebral vein

f.c.c', external carotid foramen (entrance to socket)

f.i.c., internal carotid foramen

f.m., foramen magnum

f.o.p., foramen of ophthalmicus profundus nerve (leaving the socket)

f.o.VII, ophthalmic foramen of VIIth nerve (leaving the socket)

f.r-a., foramen for ramus anastomoticus artery

f.s-m., foramen for spino-muscularis artery

f.I., foramen through which the olfactory nerve leaves the cranium

f.II, foramen for the second cranial or optic nerve

f.III, foramen for third cranial or oculomotor nerve

f.IV, foramen for fourth cranial or trochlear nerve

f.VII, foramen for seventh cranial or facial nerve

f.VIII, foramen for eighth cranial or auditory nerve

f.IX, foramen for ninth cranial or glossopharyngeal nerve

f.X, foramen for tenth or vagus nerve

i.o., interorbital canal

o.cd., occipital condyle

o.cr., occipital crest

o.f., orbital fissure

o-n., orbito-nasal canal (entrance to socket)

pl.f., palatal fossa

po.hm., posterior hyomandibular process

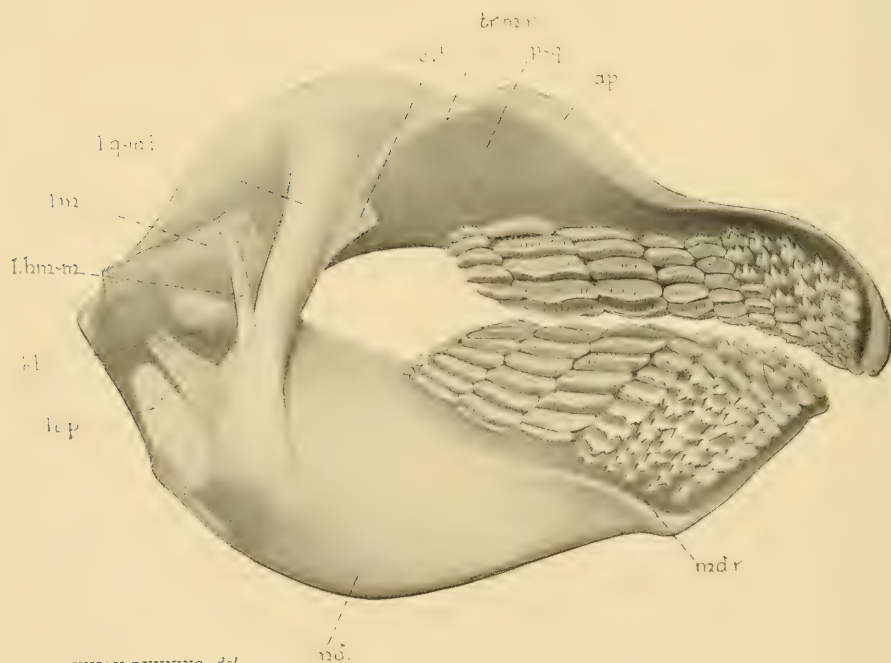
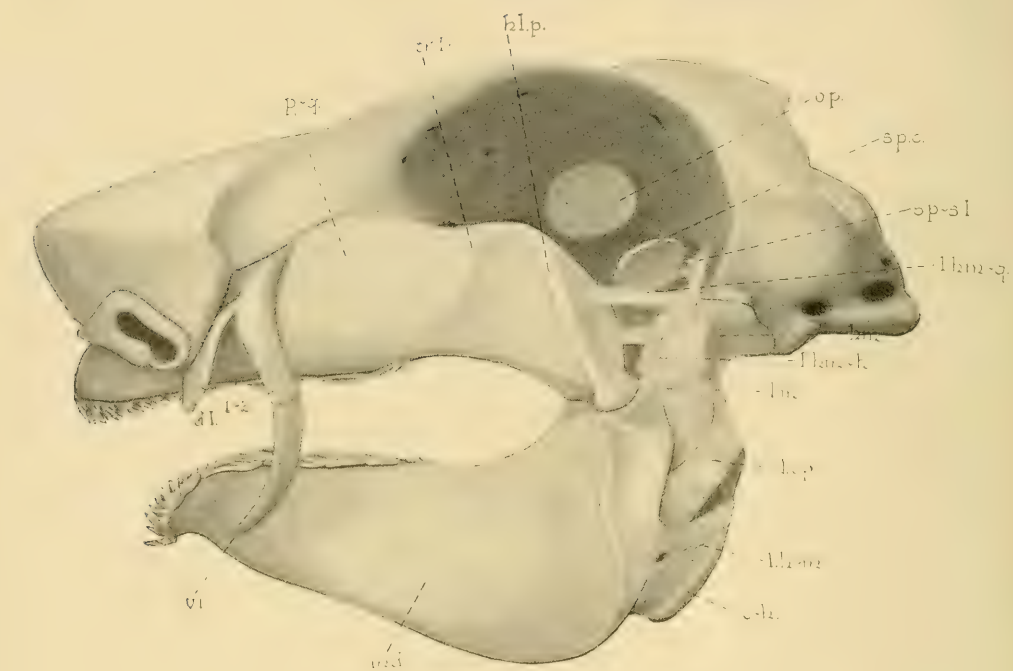
po.o., postorbital process

pr.o., preorbital process

s.hm., superior hyomandibular process

s.o., supraorbital crest

J. FRANK DANIEL



DUNCAN DUNNING, *del.*

PLATE 3

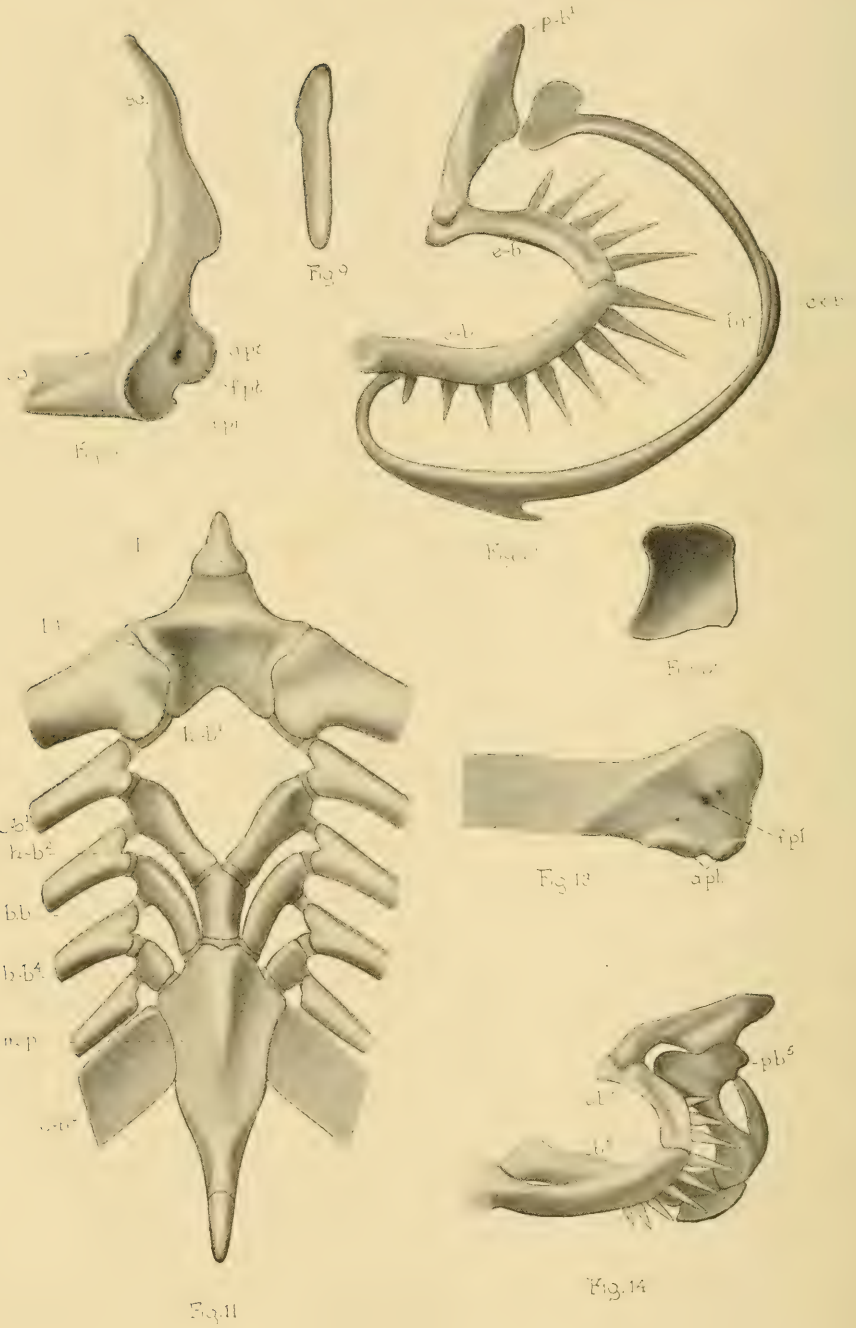
EXPLANATION OF FIGURES

Figures of *Heterodontus francisci* (natural size).

6 Lateral view of the cranium with articulation of first and second visceral arches.

7 Median view of mandibular arch showing articulation.

<i>a.p.</i> , articular process of palato-quadrate	<i>l.h-m.q.</i> , ligamentum hyomandibulo-quadratum
<i>c-h.</i> , ceratohyoid	<i>l.m.</i> , ligamentum mediale
<i>c.l'</i> , slip from palato-cranial capsular ligament	<i>l.q-m.i.</i> , ligamentum quadrato-mandibulare internum
<i>d.l. 1-2</i> , first and second dorsal labial cartilages	<i>md.</i> , mandible
<i>hl.p.</i> , hyal process	<i>md.r.</i> , mandibular ridge
<i>hm.</i> , hyomandibula	<i>o.p.</i> , optic pedicle
<i>kb.</i> , mandibular knob	<i>p-q.</i> , palato-quadrate cartilage
<i>l.cp.</i> , ligamentum complexum	<i>sp.c.</i> , spiracular cartilage
<i>l.h-m.</i> , ligamentum hyoideo-mandibulare	<i>s.p-s.l.</i> , superior postspiracular ligament
<i>l.hm-h.</i> , ligamentum hyomandibulo-hyoideum	<i>tr.l.r.</i> , transverse lateral ridge
<i>l.hm-m.</i> , ligamentum hyomandibulo-mandibulare	<i>tr.m.r.</i> , transverse median ridge
	<i>v.l.</i> , ventral labial cartilage



DUNCAN DUNNING, del.

PLATE 4

EXPLANATION OF FIGURES

All figures of *Heterodontus francisci*.

- 8 Antero-lateral view of left side of pectoral girdle (one-half natural size).
- 9 Lateral view of extrahyoid cartilage ($\times 4$).
- 10 Antero-lateral view of first branchial arch; (one-half natural size).
- 11 Ventral view of median basibranchial cartilages (natural size).
- 12 Posterior view of the spiracular cartilage ($\times 1\frac{1}{4}$).
- 13 Dorsal view of right half of pelvic girdle (natural size).
- 14 Antero-lateral view of the fourth and fifth branchial arches, with attached rudimentary sixth arch (one-half natural size).

<i>a.pl.</i> , articular process pelvic fin	<i>ex.b.</i> , extrabranhial
<i>a.pr.</i> , anterior projection of pectoral arch	<i>f.pl.</i> , foramen through pelvic girdle
<i>a.pt.</i> , articular process pectoral fin	<i>f.pt.</i> , foramen through pectoral girdle
<i>b.b.</i> , basibranchial	<i>g.p.</i> , glossal process
<i>b.h.</i> , basihyal cartilage	<i>h-b. 1-4</i> , first to fourth hypobranhial
<i>b.r.</i> , branchial ray	<i>m.p.</i> , posterior median piece
<i>c-b. 1-5</i> , first to fifth ceratobranchials	<i>p-b. 1-5</i> , first to fifth pharyngobranchials
<i>co.</i> , coracoid cartilage	<i>sc.</i> , scapula
<i>e-b. 1-5</i> , first to fifth epibranchials	

PLATE 5

EXPLANATION OF FIGURES

All figures of *Heterodontus francisci* ($\times 1\frac{1}{2}$).

- 15 Lateral view of anterior vertebrae.
- 16 Lateral view of transitional vertebrae between rib-bearing and haemal arch regions.
- 17 Lateral view of caudal vertebrae (vt. 95 and not 94 perforate).

*b.d.*², second basidorsal plate
b.v., basiventral plate
c., centrum
f.d., foramen for dorsal root of nerve
f.v., foramen for ventral root of nerve
h.a., haemal arch
h.s., haemal spine

i.d., interdorsal plate
*i.d.*², second interdorsal plate
i.v., interventral plate
r., rib
s.b.d., suprabasidorsal
*vt.*², second vertebra

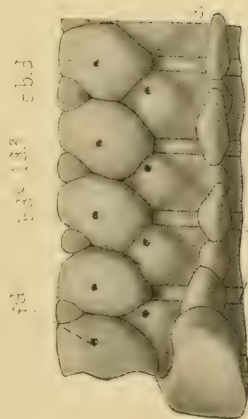


Fig. 11

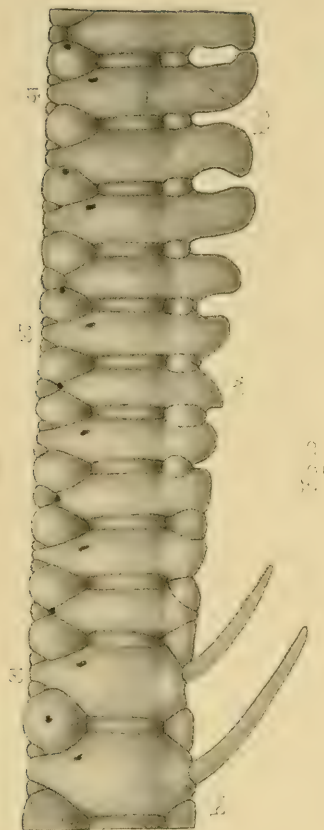
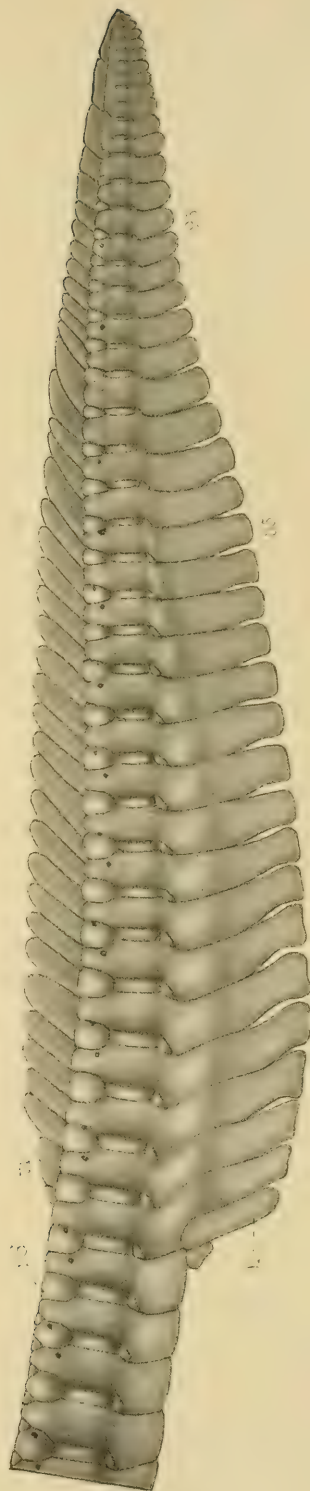


Fig. 12



DUNCAN DUNNING, *del.*

Fig. 13

PLATE 6

EXPLANATION OF FIGURES

All figures of *Heterodontus francisci* ($\times 2$).

18 End view of fifth vertebra.

19-20 Cross-section of fifth vertebra.

21 End view of fifty-third vertebra.

22-23 Cross-sections of fifty-third vertebra.

b.d., basidorsal plate

b.v., basiventral plate

c., centrum

ch., notochord

cl.p., calcified plate

cl.r., calcified ring

h.a., haemal arch

i.d., interdorsal plate

i.v., interventral plate

n.a., neural arch

r., rib

s.b.d., suprabasidorsal plate

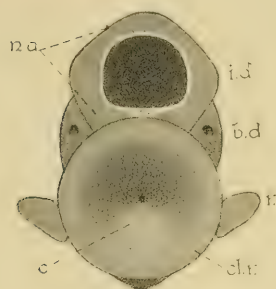


Fig. 18



Fig. 20

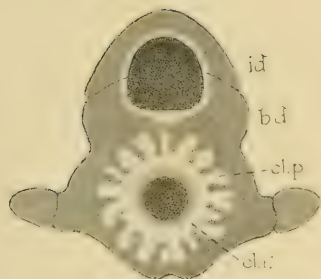


Fig. 19



Fig. 20



Fig. 22



Fig. 23

PLATE 7

EXPLANATION OF FIGURES

All figures of *Heterodontus francisci* (three-fourths natural size).

24 Lateral view of left side of first dorsal fin (female).

25 Lateral view of left side of second dorsal fin (female).

27 Dorsal view of left pelvic fin of male, showing skeleton of clasper.

b. 1-2, first and second segments following the basipterygium

b.c., basal cartilage

ba., basal piece

ba.p., basipterygium

β , beta cartilage

d.mg., dorsal marginal cartilage of clasper

d.tr. 1-2, first and second dorsal terminal cartilage of clasper

pl., pelvic girdle

ra., radial cartilage

tr. 3, accessory terminal cartilage of clasper

v.tr., ventral terminal cartilage of clasper

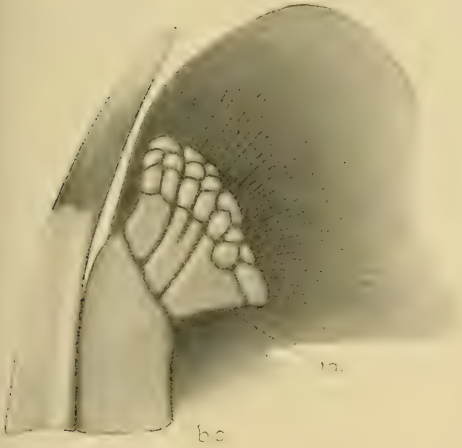


Fig. 25

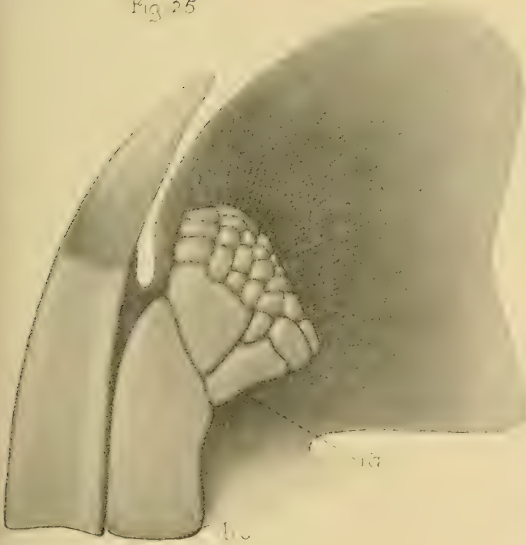


Fig. 24

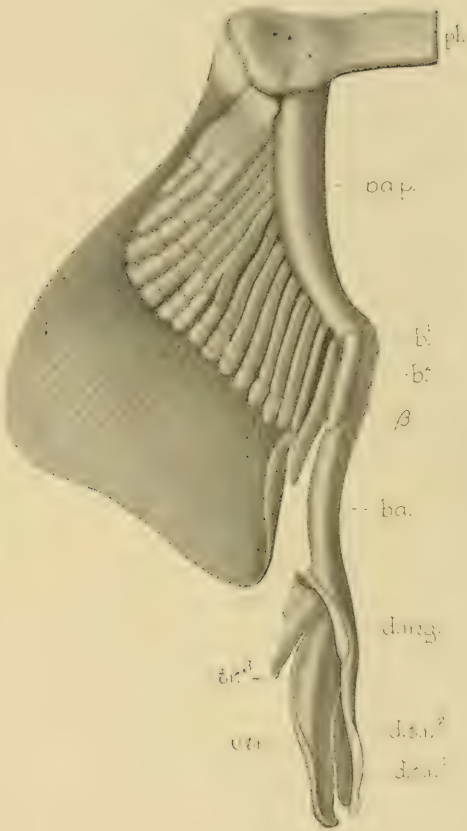


Fig. 27

DUNCAN DUNNING, del.

PLATE 8

EXPLANATION OF FIGURES

All figures of *Heterodontus francisci* (three-fourths natural size).

26 Dorsal view of left pectoral fin.

28 Lateral view of anal fin from left side.

29 Dorsal view of left pelvic fin of female.

ba.p., basipterygium

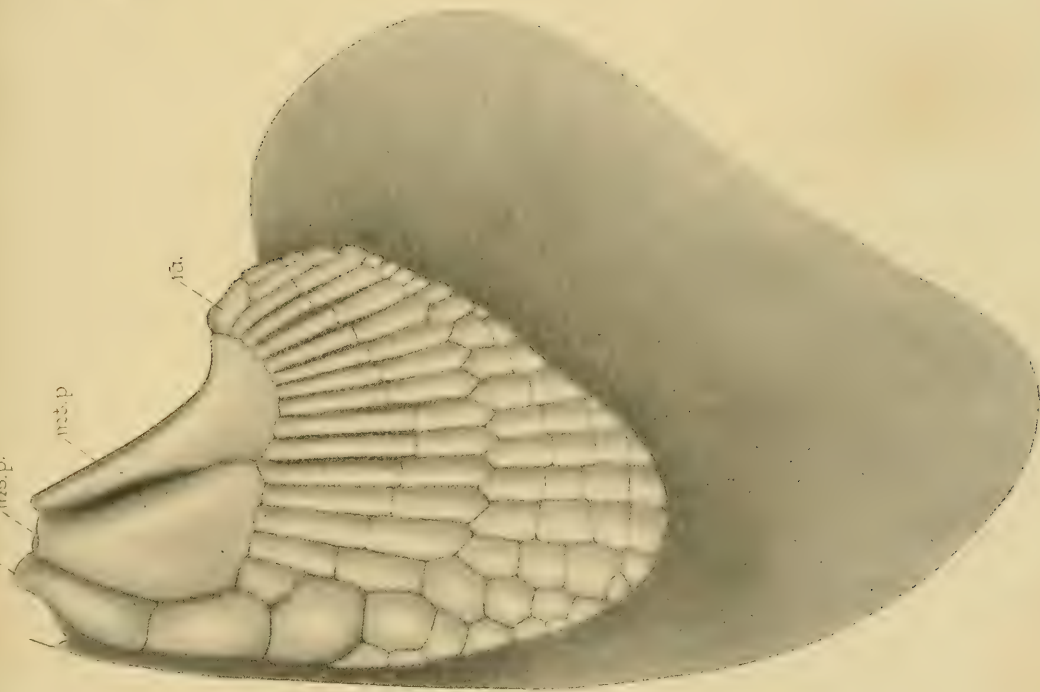
b.c., basal cartilage

ms.p., mesopterygium

ml.p., metapterygium

pr.p., propterygium

ra., radial cartilage



DUNCAN DUNNING, *del.*
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Fig. 26



Fig. 23

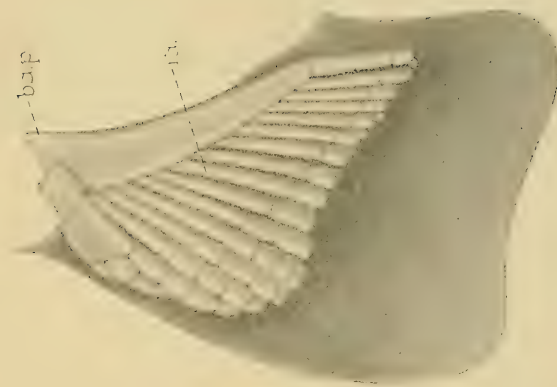


Fig. 29

STUDIES ON GERM CELLS

IV. PROTOPLASMIC DIFFERENTIATION IN THE OOCYTES OF CERTAIN HYMENOPTERA

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NINETY-EIGHT FIGURES (THIRTEEN PLATES)

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I. THE DIFFERENTIATION OF THE OOCYTES AND NURSE CELLS IN THE OVARIES OF THE HONEY-BEE, *APIS MELLIFICA*

As the writer has recently pointed out (Hegner '14 c), there are in many animals two definite periods in the germ-cell cycle during which germ cells and somatic cells arise from the same mother cells. One period occurs during embryonic development when the primordial germ cells are segregated. This segregation takes place at different stages of development in different species. For example, in the midge, *Chironomus*, one of the first four cleavage cells gives rise to all of the germ cells (Hasper '11); in the paedogenetic fly, *Miastor*, the primordial germ cell is differentiated at the eight-cell stage (Kahle '08; Hegner '14 a) but in most cases where a very early segregation has been observed, one cell at the thirty-two-cell stage is the primordial germ cell, as in *Ascaris* (Boveri '92), in *Cyclops* (Haecker '97;

Amma '11), and in *Sagitta* (Elpatiewsky '09, '10; Buchner, 10 a, '10 b; Stevens '10). The other period is that of the differentiation of the oocytes and nurse cells in the female and, at least in man, of the differentiation of the spermatocytes and Sertoli cells in the male (Montgomery '11; Winiwarter '12). These two periods seem rather distantly removed from each other, since we ordinarily begin our ontogenetic studies after the eggs are laid, but in reality they are very close together in the germ-cell cycle since the oocytes and nurse cells often become differentiated shortly before the deposition of the eggs, and the primordial germ cells are segregated shortly after cleavage begins.

This contribution deals entirely with the second period described above and the data have been derived from a study of the cellular elements in the ovaries of the queen honey-bee, *Apis mellifica*. Bees of three ages were employed: (1) those still within their pupal cells, (2) virgin queens three days old, and (3) virgin queens shortly before the deposition of eggs. The ovaries were dissected out in Ringer's solution and fixed in five different fluids: (1) Towers', (2) Carnoy's, (3) Bouin's, (4) Altmann's, and (5) Meves' modification of Flemming's solution.¹

¹ The formulae are as follows:

- | | |
|--|----------|
| (1) Tower's solution. | |
| Saturated sol. HgCl_2 in 35 per cent alcohol..... | 95 vols. |
| Glacial acetic acid..... | 2 vols. |
| Nitric acid, c.p..... | 3 vols. |
| (2) Carnoy's solution. | |
| Absolute alcohol..... | 1 vol. |
| Glacial acetic acid..... | 1 vol. |
| Chloroform..... | 1 vol. |
| HgCl_2 to saturation | |
| (3) Bouin's solution | |
| Picric acid, sat. aqueous sol..... | 75 vols. |
| Formol..... | 25 vols. |
| Glacial acetic acid..... | 5 vols. |
| (4) Altmann's solution | |
| Bichromate of potash, 5 per cent..... | 1 vol. |
| Osmic acid, 2 per cent..... | 1 vol. |
| (5) Meves' solution | |
| Osmic acid, 2 per cent..... | 100 cc. |
| Chromic acid..... | 0.5 cc. |
| NaCl | 1 gram. |
| Glacial acetic (just before using)..... | 30 drops |

The general structure of the ovary and ovarioles was obtained from in toto preparations and from thick sections. Detailed studies were made of sections 4μ thick and stained in the following ways: (1) Heidenhain's iron hematoxylin; (2) Rubaschkin's mitochondrial method;² (3) Altmann's acid fuchsin; (4) Benda's crystal violet; and (5) Delafield's hematoxylin. These methods of fixation and of staining were selected in order that both cytoplasmic and nuclear bodies could be studied.

The ovaries of insects consist of a number of tubes, the ovarioles, which are attached at the anterior end by means of terminal threads and open at the posterior end into the oviduct. The variations in the structures of the ovarioles are due principally to the presence or absence of nurse cells and the distribution of these when present. Certain ovaries, for example, those of the Orthoptera and Aptera, are not provided with nurse cells. In others the nurse cells may remain within a terminal chamber and supply the growing oocytes through a nutritive strand, as in certain Hemiptera and Coleoptera; or a rather definite number of nurse cells may become separated from the terminal chamber and accompany each oocyte, as in the Neuroptera, Hymenoptera, Diptera, and Lepidoptera. The result of the last named method of nutrition is the formation of ovarioles which resemble rows of beads.

In the bee each oocyte is accompanied by a group of nurse cells. An outline of a single ovariole is shown in figure 1. The terminal filament (*t*) consists of a row of long slender cells which extend entirely across the filament. Following this is a region occupying about half of the entire ovariole which is characterized by rosettes of cells (*r*). These cells are apparently all alike

² Rubaschkin fixes tissues in Meves' modification of Flemming's solution for one or two days. Sections are treated as follows:

Potassium permanganate, $\frac{1}{4}$ per cent.....	1 minute
Wash in water	
Oxalic acid and potassium sulphate, $\frac{1}{4}$ per cent.....	1 minute
Wash in running water.....	15 minutes
Ferric alum, 4 per cent.....	24 hours
Weigert's hematoxylin.....	2-3 days
Differentiate in ferric alum, 2 per cent	

and those in a single rosette have descended from a single mother cell which may be called the oogonial mother cell. The actual differentiation of the oocytes and nurse cells occurs in a much shorter part of the ovariole (*d*). After the oocytes are definitely established, they move down the ovariole, become arranged in a single row (*o*) and are gradually separated from each other by groups of nurse cells (*n*) which lie in nurse chambers just above them. The elements within ovarioles of different ages differ, of course, both in their stage of development and in their distribution.

The three kinds of cells within the ovaries of insects are the oocytes, nurse cells, and epithelial cells. These three kinds of cells arise differently in different groups of insects. Thus the nurse cells and epithelial cells in the paedogenetic fly, *Miastor* (Kahle '08; Hegner '14 a) are of mesodermal origin and the germ cells give rise only to oocytes. In the Hymenoptera on the other hand Korschelt ('86) in *Bombus*, Pauleke ('01) in *Apis*, and Marshall ('07) in *Polistes* agree that the three cellular elements within the ovaries arise from one sort of cells, the germ cells. I have been unable to determine the origin of the epithelial cells in the ovarioles of the bee because of the lack of young ovaries, but that nurse cells and oocytes arise from oogonia there can be no doubt.

Part of the rosette region of an ovariole is shown in figure 2. Two kinds of cells are present, (1) Those that make up the rosettes (*r*); and (2) the epithelial cells (*e*) among the rosettes. The ground substance within the ovariole in this region appears to be a loose cytoplasmic reticulum containing a few scattered nuclei. These nuclei are rather irregular in shape, and contain a clear matrix in which may be seen one or two large chromatin masses and a very delicate reticulum. No epithelial cell boundaries could be observed in this part of the ovariole and it seems probable that the rosettes are imbedded in a syncytium. There seems to be no regular arrangement of the rosettes; they do not crowd one another, but the cells in each are closely united, hence it is a very simple matter to distinguish the separate rosettes in an ovariole even with low magnification. It seems strange

because of this perfect distinctness that Pauleke ('01) failed to observe these rosettes.

The evidence for the statement that all of the cells in a single rosette have descended from a single mother cell is irrefutable. In figure 2 the cells of the rosette to which the guide line(*r*) extends are grouped about a branching strand which stains black in iron hematoxylin. A similar rosette is shown enlarged in figure 3. One branch of the black strand extends into the cytoplasm of each cell. These strands consist of the spindle fibers remaining after previous mitotic divisions, and, as will be pointed out later, such strands are not uncommon in either the ovaries or the testes of insects. A section through one end of a rosette at right angles to that shown in figure 3 is illustrated in figure 4. The spindle remains form a sort of axis about which the strands from the most recent divisions are radially arranged. The entire rosette is therefore oblong and may be sectioned longitudinally or transversely. The number of cells in each of the rosettes figured is sixteen, indicating that four divisions had occurred since the oogonial mother cell was established. No evidence was obtained which indicated the presence of amitosis in these ovarioles, and very few mitotic division figures were observed. Those that were found were invariably restricted to the cells in single rosettes (fig. 5), thus indicating that the cells in a rosette divide synchronously.

A critical examination of both the cytoplasm and nuclei of the cells in the rosettes failed to reveal any constant differences among the cells of any particular rosette. Giardina ('01) discovered a difference in the nuclei of certain rosette cells in the ovarioles of *Dytiscus*, and Kern ('12) has reported a difference in the cytoplasm of similar cells in *Carabus*, but no such distinguishing marks were found in the bee. This indicates that all of the cells at this stage in the oogenesis of the bee are probably potentially alike. At any rate no visible differences were discovered in material fixed and stained so as to bring out to the best possible advantage both nuclear and cytoplasmic bodies.

The rosette zone in the ovariole is followed by the zone of differentiation (fig. 1, *d*). Certain of the cells increase in size

and are recognizable as oocytes (fig. 6, *o*). This is brought about by an increase in the amount of cytoplasm and by the enlargement of the nucleus. The arrangement of the chromatin within the nucleus changes during this differentiation; that of the nurse cells (fig. 6, *n*) retains the condition characteristic of the rosette stage (fig. 2), whereas in the newly formed oocytes the chromatin forms threads which are scattered about irregularly within the nucleus (fig. 6, *o*). The connecting strands, so noticeable in the rosettes (fig. 3), either disappear at this time or lose their staining capacity since they are apparently absent from this stage on. Nevertheless it is very easy to determine which cells have descended from a single mother cell since a dark double ring remains where the strands passed from one cell to another (fig. 7). These rings are quite conspicuous but were completely overlooked by Pauleke ('01).

The change from the rosette zone to the zone of differentiation in the ovariole of the bee is an abrupt one—a fact which makes a study of the differentiation of the oocyte difficult, since no intermediate stages can be studied unless material in just the proper condition is obtained. In several cases which will be described later, investigators have found that a single rosette gives rise to one oocyte and a group of nurse cells. This is certainly not true in the bee, since the oocytes in the zone of differentiation are much too numerous, compared with the number of rosettes, and many instances were observed of two or more oocytes which had been directly connected by spindle remains as indicated by the presence of double rings between them (fig. 7).

If all of the cells in a single rosette are potentially alike the question arises, what causes some of the cells to become oocytes and others nurse cells? Three explanations have occurred to me: (1) There may be differential changes during the mitotic divisions in rosette formation as in *Dytiscus* (Giardina '01) resulting in one or more cells (oocytes) which differ in content from the others (nurse cells). No visible changes of this sort were observed. (2) The polarity of the rosette may influence the cells in such a way that those near the center of the ovariole and closest to the zone of differentiation tend to develop into

oocytes. (3) Those cells of the rosette which reach the zone of differentiation first are stimulated to become oocytes and by their growth and differentiation prevent the other cells of the rosette from similar changes. It would be futile to argue on the basis of known facts in favor of any of these hypotheses.

The arrangement of the oocytes and nurse cells within the ovariole resulting in a linear series of oocytes which alternate with groups of nurse cells takes place a short distance back of the zone of differentiation (fig. 1, *n*). Paulcke ('01) has satisfactorily described and figured the formation of the epithelium around the oocytes and the structure of the nurse chamber, but, as stated above, he failed to see the intercellular rings which indicate the descent of the cells concerned. A group of nurse cells about an oocyte is shown in figure 8. This oocyte is connected with at least three nurse cells. One of the nurse cells (*a*) lies below the oocyte in the ovariole; since this is never true in later stages it is probable that such a cell would either degenerate or become separated from the oocyte and forced over to one side. This has evidently happened in the case of the oocyte illustrated in figure 9, since a ring is present here at the lower end (*a*), but it does not connect the oocyte with a nurse cell. The relation between the oocyte and its accompanying nurse cells is shown in figure 10. All of the nurse cells are not included, since this is a camera drawing of a section. It illustrates, however, the way in which the nurse cells form into rows converging toward the oocyte.

The descent of the cells within the zone of differentiation would be impossible to determine if it were not for the presence of the rings between them. These rings continue to connect the nurse cells with the oocyte, even in late stages in the growth of the latter (fig. 11) and many of them may also persist between the nurse cells after the nurse chamber is fully formed, as in the stage illustrated in figure 12. Kern ('12) also finds these rings connecting the nurse cells with the oocytes of *Carabus*, and claims that nutritive material passes through them during the growth of the egg.

As soon as the oocytes are differentiated, numerous granules of various sizes appear within their cytoplasm; in the earlier

stages these lie mostly near the nucleus (figs. 7-8), but later (fig. 9) become scattered throughout the cytoplasm. These granules stain best in iron hematoxylin after fixation in Meves' modification of Flemming's solution. No evidence was obtained that they are of nuclear origin, although their early position near the nucleus indicates that they may have arisen in this way; or if not directly from the nucleus, at least through its influence. On the other hand, their sudden appearance within the cytoplasm indicates that they are cytoplasmic bodies which have resulted either from the aggregation of smaller pre-existing bodies of a similar nature or from the synthesis of other substances under the stimulus of the metabolic processes set up at the inauguration of the growth period. Duesburg ('08) has recognized granules in the peripheral layer of cytoplasm in the full grown egg of the bee, especially near the nucleus in the thickened area which Petrunkevitch ('01) has called the 'Richtungsplasma,' and considers them to be mitochondrial in nature. It seems probable that the bodies we have observed are the 'mitochondria' of Duesburg at an earlier stage. Paulcke ('01) failed to observe them.

Discussion. The differentiation of the cellular elements in the ovaries of insects and the relations of the oocytes to the nurse cells has interested students of histology and cytology for three quarters of a century. Mayer, as early as 1849, expressed the opinion that the nurse cells are abortive eggs. The connections between them and the oocytes were observed by Huxley ('58) in oviparous aphids, and were considered by him a nutritive canal for the conduction of food material from the nurse cells to the growing egg—a conclusion concurred in by Lubbock ('60) and Claus ('64). Balbiani ('70), however, proved this 'nutritive canal' to be a protoplasmic strand, but, as Wielowiejski ('85) has pointed out, he was in error when he stated that the terminal chambers of the ovarioles of aphids contain a large central cell which gives rise to both the oocytes and nurse cells (abortive eggs). He nevertheless established the fact of a protoplasmic cellular bridge between these two kinds of cells.

Protoplasmic bridges between the cells of Metazoa are not uncommon and may exist in all tissues. As a rule, they are delicate strands which pass through pores in the cell walls. The cellular elements in a syncytium, such as occurs during the cleavage of the insect egg, must be even more closely united physiologically, since here the cytoplasm forms a continuous network. Cellular bridges similar to those described above in the queen bee, have been observed in the germ glands of a number of other animals, especially insects, but mostly during spermatogenesis. Thus Platner ('86) found in Lepidoptera that often two neighboring spermatocytes, and sometimes three, were connected by intercellular ligaments which were attached to an intracytoplasmic body in each cell. The latter were considered 'Nebenkerne.' Similar conditions were discovered by Prenant ('88), Zimmerman ('91) and Lee ('95) in the male germ cells of Gastropoda. Lee, in his work on *Helix*, recognized the true origin of the intercellular bridges and their significance. They were found to be the remains of the spindle fibers following a mitotic division. The term 'pont fusorial' was applied by Lee to the bridge itself and 'moignons fusoriaux' to the ramification of the fibers within the cytoplasm of the cells. Similar intercellular ligaments were observed by Henneguy ('96) in the seminal cells of *Caloptenus*; by Erlanger ('96, '97) in both the testes and ovaries of the earthworm; by Wagner ('96) in the male germ cells of spiders; by Meves ('97) in both the testes and ovaries of the salamander; by Giardina ('01), Debaisieux ('09) and Günthert ('10) in the ovaries of *Dytiscus*; by Marshall ('07) in the ovarioles of *Polistes*; by Kern ('12) in the ovarioles of *Carabus*; by Govaerts ('13) in the ovarioles of *Carabus* and *Cicindela*; by Maziarski ('13) in the ovarioles of *Vespa*; and by Hegner ('14 a) in the testes of *Leptinotarsa*.

By far the most interesting results are those obtained by Giardina and confirmed by Debaisieux ('09) and Günthert ('10). Giardina proved conclusively that a single oogonium in the ovary of *Dytiscus* undergoes four divisions, thus producing sixteen cells, one of which is the oocyte and the remaining fifteen nurse cells. The processes of differentiation in this genus are partic-

ularly interesting, because they include a separation of the chromatin of the mother cell into two masses. One of these masses of chromatin forms an 'anello cromatico;' the other gives rise to forty chromosomes which divide equally, half of each passing to each daughter cell. The chromatic ring remains undivided and becomes situated entirely in one of the daughter cells. At each of the three succeeding divisions the chromatic ring is segregated entirely in one cell; this cell is the oocyte, whereas the other fifteen which have a common origin with it are nurse cells.

Since the publication of Giardina's observations many investigators have attempted to discover similar visible differentiations in the ovaries of other insects, but without much success. Thus Govaerts ('13) made detailed studies of beetles of the genera *Carabus*, *Cicindela*, and *Trichisoma* but was unable to find anything resembling the chromatic ring which occurs in *Dytiscus*. He found however that the spindle fibers ('residu fusorial') persist after the daughter cells are formed during the differential divisions, just as they do in *Dytiscus*, and that a definite polarity is marked by the position of these spindle remains. The conclusion is reached that something more fundamental than the unequal division of chromatic elements is responsible for the differential divisions and decided in favor of a 'polarite predifferentielle.' No explanation is offered, however, as to the origin of this polarization.

A brief account of the oogenesis in carabid beetles has also been published by Kern ('12), who finds that during the differential mitoses, the oocyte mother-cell may be distinguished by the presence of certain intracytoplasmic granules which he describes as follows:

Befinden sich die Zellen der Zellrosetten in Teilung, so findet man mitunter in einer Zelle neben der Teilungsfigur eine Anhäufung von färbbaren Körnchen, ähnlich denjenigen, die in späteren Stadien in der jungen Eizelle im Cytoplasma gefunden werden. Es liegt nahe, an einen *Diminutionsvorgang*, ähnlich demjenigen, welchen Giardina bei *Dytiscus* beschrieben hat, oder auch an einen Vergleich mit den *Ectosomen* bei *Cyclops* zu denken; doch gelang es mir bisher nicht, alle Einzelheiten festzustellen. Die Körnchen im Cytoplasma junger Eizellen werden nach und nach aufgelöst.

The origin of these granules was not determined, and although Kern is inclined to consider them similar to the chromatic-ring substance in *Dytiscus*, there is a possibility that they may be mitochondrial in nature or may consist of some other cytoplasmic material.

The presence of intercellular bridges is important, since it makes it possible to determine the relationship of the groups of cells in the ovarioles. But in the queen bee these bridges do not persist to any considerable extent after the zone of differentiation has been reached. Here, however, as shown in figures 7 to 12, there are well defined rings between the cells which indicate their relationship. It might be argued that these rings may arise where two cells happen to come into contact, if it were not for the fact that all stages between the fully developed bridges and the presence of clearly defined rings have been observed. These are no doubt the persisting mid-bodies or 'Zwischenkörper' which remain between the cells after division. They have been noted especially by Giardina ('01) in *Dytiscus*; by Marshall ('07) in *Polistes*; by Kern ('12) in *Carabus*; and by Maziarski ('13) in *Vespa*.

Summary of Part I. 1. Four rather definite regions may be recognized in the ovariole of the queen honey bee (fig. 1): (a) the terminal filament; (b) a rosette region; (c) a zone of differentiation; and (d) the posterior part in which the oocytes are arranged in a linear series and separated from each other by groups of nurse cells.

2. The rosette region is filled with rosette-like groups of cells, each group consisting of the descendants of a single mother oogonium. The cells of a rosette are united by strands which are the persisting spindle fibers from earlier mitoses (fig. 3). The cells in a rosette divide synchronously (fig. 5).

3. Oocytes and nurse cells are both derived from the oogonia. Their differentiation occurs in the zone of differentiation (fig. 1, d). One or more cells of each rosette enlarges and becomes an oocyte, whereas the others retain more of their earlier characteristics and become nurse cells. Although the strands which connected the cells in a rosette disappear, the descendants of a single oogonium

may still be determined, because of the presence of deeply staining rings between the cells (figs. 7-12).

4. The causes of differentiation could not be definitely determined, but several hypotheses are mentioned (p. 500).

5. Granules appear near the nucleus of oocytes shortly after their differentiation. Later they become distributed throughout the egg cytoplasm. These granules appear to be mitochondrial in nature and to arise from, or under the influence of the nucleus.

II. THE BACTERIA-LIKE RODS AND SECONDARY NUCLEI IN THE OOCYTES OF *CAMPONOTUS HERCULEANUS* VAR. *PENNSYLVANICA* DEG.

The important contributions by Blochmann ('84, '86) upon the growth of the oocytes in ants seem to be the only reports that have ever been made on this subject. Blochmann discovered two very interesting facts regarding these oocytes: (1) the presence of rod-shaped bodies almost completely filling the growing egg which he considered symbiotic bacteria, and (2) the formation of nuclear-like bodies around the oocyte nucleus. Recently Tanquary ('13) has described, in the freshly laid eggs of the carpenter ant, a body which he calls a cleavage nucleus, but which resembles very closely bodies that have been discovered in the eggs of other animals and to which I have applied the term *keimbahn-* or *germ-line determinants*. The observations recorded in the following pages were made in order to trace the genesis of the eggs of ants with special reference to the origin, distribution, and fate of the bacteria-like bodies, nuclear-like bodies, and the *germ-line determinants*.

The material used for these studies consisted of the ovaries of the carpenter ant, *Camponotus herculeanus* var. *pennsylvanica* DeGeer. A large number of virgin queens were obtained from a dying apple tree on April 3, 1914, and some of them were kept alive until June 9, 1914. The ovaries were dissected out in Ringer's solution and immediately fixed in the same manner as were those of the honey bee (page 496). Ovaries were preserved at intervals of a few days during the period of two

months. In this way oocytes in all stages of growth were obtained up to almost the period of deposition. Sections were cut and stained as in the queen bee.

The ovaries of the carpenter ant resemble those of the queen bee in general structure and the ovarioles are likewise similar. The youngest ovaries obtained had already passed the period when the oocytes and nurse cells are differentiated, so there was no opportunity to study the events that occur during this differentiation. Four regions may be distinguished in the ovarioles as shown in figure 13. There is a terminal filament (*t*) at the anterior end. This is followed by a region which we may call the terminal chamber (*t.c.*) containing oocytes, nurse cells, and epithelial cells without any special arrangement. The next part of the ovariole is short and contains oocytes which have grown considerably but have not yet taken a position in the axis of the tubule. This we may call the first zone of growth (*g*). The rest of the ovariole consists of a linear series of oocytes (*o*) each with its accompanying group of nurse cells (*n*). Each oocyte is larger than the one anterior to it and the nurse cells gradually become grouped into a definite nurse chamber (*n.c.*). The bacteria-like bodies discovered by Blochmann are present only in the last described zone. The first signs of nuclear-like bodies around the oocyte nucleus also appear here. For the sake of convenience oocytes in the various stages which need to be referred to have been drawn in outline and to scale as shown in figures 14 and 15.

The posterior end of the terminal filament (*t*) and anterior end of the terminal chamber (*t.c.*) are shown in outline in figure 16. The cells of the terminal filament are long and slender and extend entirely across it. One is shown enlarged in figure 17. Within the terminal chamber are two kinds of cells, oocytes and nurse cells. The oocytes, as indicated in figure 18, are the youngest to be found in the ovarioles at this time and I have regarded them as Stage A (fig. 14). The cell walls of the nurse cells are not very distinct. Their nuclei (fig. 19) are much smaller than those of the oocytes and contain a single irregular mass of chromatin granules. The structure of the oocytes and nurse cells is similar throughout the entire terminal chamber.

The terminal chamber is separated from the first zone of growth (fig. 20) by what appears to be a distinct membrane (*m*). The condition of all of the oocytes is similar throughout this zone (Stage B, fig. 14). The oocytes have grown considerably and their nuclei (fig. 21) contain a few clumps of chromatin granules lying near the nuclear membrane. Outside of the nucleus (fig. 21) is a layer of darkly staining substance which resembles chromatin in some respects and may represent chromatin which has passed through the nuclear membrane into the cytoplasm. The nurse cells now have definite cell walls (fig. 22) and are also characterized by a layer of darkly staining material lying around the nucleus. Among the oocytes and nurse cells are a few epithelial cells (fig. 23); these have no definite cell walls, and their nuclei are rather irregular in shape and contain a single mass of chromatin.

Whether or not the first zone of growth is definitely separated from the remaining part of the ovariole could not be determined with certainty, but its limit is conspicuously marked by the abrupt appearance of the bacteria-like bodies of Blochmann. This is indicated in figure 24, which shows the posterior portion of the first zone of growth and the anterior part of the rest of the ovariole. In the upper part of this figure is a single oocyte in Stage B and a number of nurse cells. These are apparently embedded in a loose reticulum of cytoplasm. Further down the ovariole the spaces surrounding the nurse cells and epithelial cell nuclei are filled with more or less wavy rods which Blochmann considered symbiotic bacteria. These rods extend throughout the ovariole in all directions, being represented by distinct spherical granules where cut across.

From this point on, the oocytes are arranged in a linear row in the central axis of the ovariole (figs. 13 and 25). The cytoplasm of the oocytes increases rapidly in amount, but the nuclei enlarge very little. The nurse cells (fig. 25, *n*) become arranged more or less definitely into rows which radiate toward the upper end of the oocyte. Those nurse cells closest to the oocyte increase more rapidly in size than do the others. Compare, for example, that lettered *a* in figure 25 with its companions, and

those accompanying the upper oocyte with those of the lower oocyte. Surrounding the oocytes, nurse cells, and epithelial cell nuclei are the groups of bacteria-like bodies.

The transition of the oocyte from Stage C (fig. 14, *C*; fig. 25, *C*₂) to Stage D (fig. 14, *D*, fig. 26) is accompanied by an invasion of the oocyte cytoplasm by the bacteria-like rods. Some of these rods form almost perfect circles, resulting in what at first sight appear to be vacuoles. Some of the epithelial-cell nuclei are in very close contact with the oocyte but these were not observed actually within the oocyte cytoplasm.

The principal difference between an oocyte in Stage D (fig. 26) and one in Stage E (fig. 14, *E*; fig. 27) is the sudden appearance of nuclear-like bodies around the nucleus, which I shall call secondary nuclei. The nucleus itself is about equal in size to that of the preceding stage (fig. 26). The chromatin, which in younger oocytes (figs. 24–26) has gradually migrated from the periphery toward the center of the nucleus where it formed an irregular clump, has again become scattered, being represented by a few smaller and widely separated masses. In the illustration (fig. 27) three secondary nuclei are shown lying below but in contact with the oocyte nucleus. These likewise contain a delicate reticulum and from one to three chromatin masses. No intermediate stages between the nucleus of Stage D (fig. 26) and that of Stage E (fig. 27) were discovered, and it was thus impossible to determine with certainty the origin of these secondary nuclei. If, however, the oocyte nucleus continued to increase in size at the same rate as indicated in Stage C (fig. 25) and in Stage D (fig. 26) it would be about the size of that in figure 27 *after* having given rise to the secondary nuclei by the method of budding or in some other way. This subject will be discussed more in detail later.

During the interval between Stage E (fig. 14, *E*; fig. 27) and Stage F (fig. 14, *F*, fig. 28) the oocyte enlarges until it extends almost across the ovariole, and the epithelial cell nuclei become arranged in a single layer around it, forming a follicle. At this time (fig. 28) the cytoplasm of the oocyte and that surrounding the nurse cells and epithelial-cell nuclei is crowded full of the

bacteria-like rods. The secondary nuclei also increase in number around the oocyte nucleus; the nucleus itself does not increase in size. Both the oocyte nucleus and the secondary nuclei are sometimes irregular in shape, a condition that may be due to the effects of fixation, or that may represent a stage in budding or in amitotic nuclear division (page 518).

The next phase of the growth period (Stage G, fig. 14, *G*, fig. 29) witnesses the lengthening of the oocyte and the further arrangement of the nurse cells to form a compact group, which becomes surrounded by epithelial cells, thus producing a definite nurse chamber. The bacteria-like bodies increase in number as the oocyte grows and continue to fill it completely with bundles of rods. The secondary nuclei near the oocyte nucleus also increase slightly in number.

Shortly after this condition is reached the oocyte is invaded just beneath the nurse chamber by an influx of cytoplasm elaborated by the nurse cells (fig. 30, *c*). This cytoplasm is free from the bacteria-like bodies and it seems very probable that it either forces these rods out of its path or else dissolves those which it encounters. There is evidence that, from this stage on, the number of bacteria-like rods does not increase, the rods gradually lose their compact grouping and become further separated from one another, the spaces between them probably being occupied by the cytoplasm added to the oocyte by the nurse cells. The oocyte nucleus by this time (fig. 30) is completely surrounded by secondary nuclei from which it differs in appearance. The secondary nuclei contain a rather dense reticulum and one or several large chromatin granules, whereas the oocyte nucleus is very irregular in shape and contains a delicate reticulum which causes it to appear clearer. The irregular shape of the oocyte nucleus is probably due to the pressure upon it of the secondary nuclei which surround it. Its decrease in size is also noticeable and one cannot but suspect that this decrease is directly related to the increase in the number of secondary nuclei. A transverse section through an oocyte near the nurse chamber is shown in figure 31.

The nurse chamber is now completely formed (fig. 13, *n.c.*). The nurse cells are still free from the bacteria-like rods and their nuclei, as pointed out by Blochmann ('86), possess very thick membranes (fig. 30, *n*). Part of one of these nuclei greatly enlarged is shown in figure 32. The membrane contains, in a homogeneous matrix, a number of vacuoles and a great many granules of various sizes which appear in material fixed and stained by a number of different methods. Their reactions all indicate that they are chromatic in nature and their position suggests that they may have migrated into the membrane from inside of the nucleus and are on their way into the cytoplasm. It could not be definitely determined, however, whether this is a true case of chromatin emission or simply a condition due to the action of the fixing solutions used.

A further increase in the amount of cytoplasm within the oocyte is evident when Stage H (fig. 15, *H*; fig. 33) is reached. Here an opening (*a*) is present in the follicle connecting the oocyte directly with the nurse chamber. The small plug of cytoplasm filling this channel is no doubt homologous with the nutritive string present in the ovarioles of insects whose oocytes are not accompanied by a group of nurse cells, but are connected with the terminal chamber by a protoplasmic thread. In this stage the oocyte nucleus (*o*) is still closely pressed by the secondary nuclei (*s*) surrounding it and the entire group lies within the cytoplasmic zone. Such a group is shown enlarged in figure 34, in which the oocyte nucleus may be distinguished from the secondary nuclei by its irregular shape, central position, and clearness.

The succeeding stages in the growth of the oocyte (fig. 15, *I*, *J*, *K*, *L*; figs. 35-39) are characterized by a decrease in the number of bacteria-like rods, by the formation of yolk globules, and by the increase in number and the scattering of the secondary nuclei. Part of a section through an oocyte of Stage I (fig. 15, *I*) is shown in figure 35 which represents a portion extending from a point midway between the two poles out to the middle of the oocyte. Just within the follicular epithelium (*e*) is the suggestion of a clear layer (*k*) which later becomes the 'Keimhautblastem.' The

black spherical bodies are yolk globules (*y*) which appear to originate near the periphery and gradually to migrate into the central region. The bacteria-like rods are still present but they are widely scattered and faintly staining.

By the time the next stage is reached (fig. 15, *J*; fig. 36) the bacteria-like rods have completely disappeared everywhere except near the periphery, around the lower part of the oocyte. According to Blochmann ('86) they are still present in this region after the eggs are laid, and they are also mentioned by Tanquary ('13) in the freshly deposited eggs. The latest oocyte studied by the writer is Stage L (fig. 15, *L*), which is considerably younger than the fully grown egg. A few faintly staining rods still exist at this stage near the posterior end.

The compact group of secondary nuclei which surround the oocyte nucleus up to this stage now breaks up, and the individual nuclei become scattered throughout the entire egg near the periphery. Quite a number of them still appear near the anterior pole of an oocyte in Stage K (fig. 15, *K*; fig. 37) where they surround the opening into the nurse chamber. At some distance back of this pole the secondary nuclei are imbedded in the cytoplasm especially near the periphery. They retain at this time (Stage K) the characteristics noted in early stages; i.e., they are more or less spherical, filled with a reticulum, and contain one or several large chromatin granules (fig. 38). Later (Stage L, fig. 15, *L*; fig. 39) they seem to be more numerous and a single egg must contain hundreds of them. In this, the last stage examined, these secondary nuclei have changed in appearance as indicated in figure 40. The chromatin granules have become aggregated into a few irregular strands, a condition which may be a phase of degeneration or, as Loyez ('08) believes, a stage in the formation of yolk globules. The fate of the secondary nuclei was not discovered and, so far as I know, none of the investigators who have described similar bodies has been able to determine with certainty what becomes of them.

The posterior ends of the older oocytes in my series were carefully examined with a view to tracing the origin of the body which Tanquary ('13) observed near the posterior pole of freshly

laid eggs of *Camponotus herculeanus* var. *ferrugineus* Fabr. and called the cleavage nucleus (fig. 41, *n*). This body is obviously not a cleavage nucleus since it is not in the usual position occupied by this nucleus, and does not possess the characteristics of a cleavage nucleus. Furthermore, it persists during the early cleavage stages at the posterior end, whereas the cleavage cells (nuclei) are shown by Tanquary in their proper position near the anterior pole (fig. 42, *cc.*). It seems probable therefore that this body belongs to the class of substances which have been found in the eggs of many different kinds of animals and which later become part of the material within the primordial germ cells—substances to which I have applied the term 'Keimbahn or germ-line determinants.' This seems all the more probable since it persists at least until a late cleavage stage (fig. 43, *rn.*) and later there is present a group of cells (fig. 44, *kc.*) which Tanquary describes as a "group of small cells applied to the posterior end of the inner peripheral protoplasm," and which further research will doubtless prove to be germ cells. No bodies were discovered in my material that could be recognized as an early stage in the formation of the 'cleavage nucleus' described and figured by Tanquary.

Discussion. No opportunity was afforded by the material in my possession for determining the differentiation of the oocytes from the nurse cells, since these two sorts of cells are established in ovarioles younger than those in the virgin queens collected in the spring. The problem of the separation of germ cells (oocytes) from somatic cells (nurse cells) therefore could not be solved. The most interesting phenomena exhibited by the ovarioles are (1) the presence of the bacteria-like rods and (2) the formation and distribution of the secondary nuclei.

The bacteria-like rods. Blochmann ('84, '86) was the first to observe the bacteria-like rods in the ovarioles of two species of ants, *Camponotus ligniperda* and *Formica fusca*. Recently Tanquary ('13) has observed these bodies in the cytoplasm at the posterior end of freshly laid eggs of *Camponotus herculeanus* var. *ferrugineus*. Blochmann found those in the eggs of *Camponotus* to be from 10 to 12 μ in length, whereas those in *Formica*

were only from 4 to 5μ long and were not so regularly arranged in bundles as in the former species. He at first supposed these bacteria-like bodies to be cytoplasmic structures, but, after observing them in various stages of division, expressed the opinion that they are symbiotic bacteria.

Bodies of a similar kind have been observed in many other insects. Those that occur in the cockroaches most closely resemble the bacteria-like rods in the ovarioles of ants. These likewise were first discovered by Blochmann ('87, '92) in *Periplaneta orientalis*. They occurred not only in the eggs but also among the blastoderm cells and in the spaces formed by the liquefaction of the yolk in the embryos. Later they were observed in the anlage of the fat body where they persist in the adult stage. Wheeler ('89) described them in the 'Keimhautblastem' of *Blatta germanica* as "minute rod-shaped bodies so numerous in the surface protoplasm as to make it appear reticulate. They look like bacillar micro-organisms and stain deeply."

Mercier ('07) has subjected these bacteria-like rods in *Periplaneta orientalis* to careful study. He agrees with Blochmann regarding their distribution and confirms Blochmann's statement that they multiply by division. Mercier was able to cultivate the rods and concludes that they are true bacteria and thinks them to be of a symbiotic nature although he was unable to suggest any advantage that the host receives because of their presence. They are given the name *Bacillus cuenoti* by Mercier.

Many other investigators have reported bacteria within the eggs or tissues of insects. Blochmann ('87) observed them in *Pieris*, *Musca*, and *Vespa*; Stuhlmann ('86) shows them in many of his figures, and Forbes ('91) found them in the caecal glands of various Heteroptera. The 'green or yellow granular mass' described by Leydig ('50) in the embryos of viviparous aphids and later called the 'pseudovitellus' by Huxley ('58) and the 'green body' by Witlaczil ('84) is considered now to be due to symbiotic organisms. Of particular importance are the contributions of Mercier ('07) on the cockroach and of Sulc ('06, '10), Pierantoni ('10), and Buchner ('12) on the Hemiptera. Buchner ('12) has given a full historical discussion of the subject

besides adding considerable new material, and any one desiring a comprehensive review of the present state of our knowledge of these symbiotic organisms is referred to his paper. Thirty-four species are described and figured by Buchner. Some of them are bacteria, such as those in the cockroach, but others are more like yeasts. The infection of the egg, which reminds one of the infection of the egg of the Texas fever tick by *Piroplasma bigeminum*, may be diffuse, as in the cockroach, or localized, as in the aphids. Buchner decides that these organisms are symbiotic, but, like Mercier, was unable to discover any advantage to the insect host from the relationship.

The secondary nuclei. One of the most interesting features of the growth of the oocyte in certain insects is the formation of small nuclear-like bodies around the oocyte nucleus. Bodies of this sort were first described by Blochmann ('84, '86) in Hymenoptera. Since then they have been observed in insects belonging to this order by Stuhlmann ('86) and Marshall ('07) and similar bodies were noted by Korschelt ('86) near the nuclei of both the oocyte and nurse cells of the fly, *Musca vomitoria*. Korschelt was unable to determine the origin, function, and fate of these 'helle Bläschen' but noted their resemblance to those discovered by Blochmann.

The two ants, *Camponotus ligniperda* and *Formica fusca*, and the wasp, *Vespa vulgaris*, were all found by Blochmann to be very much alike so far as the growth of their oocytes is concerned. The origin of the nuclear-like bodies is described in *Camponotus* as follows:

Bei etwas älteren Eiern beginnt nun an der Oberfläche des Kernes ein Knopfungsprocess, der schliesslich zur Entstehung einer grossen Anzahl kleiner Kerne führt. Man bemerkt als erste Andeutung dieses Processes kleine, helle, rundliche Gebilde, die dicht an der Oberfläche des Kernes anliegen, und die ich in meiner vorläufigen Mittheilung als 'knötchenförmige Verdichtungen' bezeichnet hatte. Ich neige jetzt zu der Ansicht, dass es von vornherein kleine Vacuolen sind, da die Kernmembran sich meist etwas färbt und stets sehr scharf erscheint, während ich an diesen kleinen Gebilden bei ihrem ersten Auftreten keine derartige Membran unterscheiden könnte. Bald tritt in diesen Vacuolen ein kleines mit Pikrocarmin sich färbendes Körnchen auf diese Vacuolen oder, wie ich sie jetzt nach dem Auftreten des Chro-

matinkörnchens nennen will, Nebenkerne, nehmen allmählich an Grösse zu, wobei sie dann eine sehr deutliche Membran an ihrer Oberfläche erkennen lassen, zugleich nimmt der Inhalt an festen, färbbaren Substanzen zu. Diese treten theils als kleine, rundliche Nucleolen, oder als feine, wenig sich färbende Fädchen auf.

Da nach und nach immer mehr solche Nebenkerne entstehen, finden wir bei etwas weiter in der Entwicklung fortgeschrittenen Eiern in der Region der Eiröhre, wo bereits Eifächer und Nährzellenfächer deutlich abgegrenzt sind, die Oberfläche des Eikernes von einer ganzen Schicht solcher Nebenkerne von verschiedener Grösse bedeckt, die sich gegenseitig berühren. So bleiben die Verhältnisse auch in noch etwas älteren Eiern. (pp. 144–145).

These Nebenkerne, according to Blochmann, after multiplying by self-division become scattered within the yolk where they degenerate, none being present in the ripe egg.

Among the other investigators who have observed similar bodies in the oocytes of insects are the following: Will ('84) and Ayers ('84) observed them in Hemiptera and considered them follicular epithelial cells which contributed to the formation of the yolk. Stuhlmann ('86) has described them in many insects, including *Musca*, *Periplaneta*, *Locusta*, *Pieris*, *Aphrophora*, *Sphinx*, and certain Coleoptera and Hymenoptera. They were called 'Reifungsballen' by him and were thought to be similar to the polar bodies which at that time had not yet been observed in insects. The 'Reifungsballen' appear at different stages of the growth period in different species and also have different fates; some of them fuse to form a large 'Dotterkern' which lies near the posterior end of the egg and resembles what I called 'Keimbahn-determinants,' and others become widely distributed and disappear in the yolk. The possible origin of the 'Ballen' from epithelial cells is suggested but not considered probable. Korschelt ('89) from a study of them in *Bombus*, concludes, as did Will and Ayers, that they are derived from epithelial cells.

In *Blatta germanica* and *Leptinotarsa decemlineata*, Wheeler ('89) has described as 'maturation spheres' a number of globular bodies which appear after the egg nucleus migrates to the periphery and prepares for maturation. In *Blatta* several of these spheres may be present. In *Leptinotarsa* a number of oval

hyaline masses likewise occur which are considered the equivalents of the 'maturation spheres' in *Blatta* and homologous to the 'Reifungsballen' of Stuhlmann. No chromatin masses were observed in any of these spheres, but in *Leptinotarsa* the wandering of part of the chromatin into the yolk, where it disappears, is described. As Stuhlmann pointed out, these spheres may appear in different species at different stages in the growth period and it seems therefore possible that the 'Nebenkerne' of Blochmann, the 'Reifungsballen' of Stuhlmann and the 'maturation spheres' of Wheeler may be homologous, although the first two contain chromatin whereas the 'maturation spheres' do not. Lameere ('90) was able to confirm Blochmann's account regarding the origin of the Nebenkerne in *Camponotus* and Henneguy ('04) found them in both the wasp and the honey-bee. In the former they appear around the germinal vesicle and disappear very early, but in the bee they seem to be derived from follicular epithelial cells and persist until a later developmental stage. None of these bodies could be found in the oocytes of the honey-bee which I have studied.

Marshall ('07) made a careful study of the secondary nuclei in *Polistes*, but, like previous investigators, was unable to determine definitely regarding their origin and fate. He agrees with Blochmann that they probably arise from the germinal vesicle by budding, but was unable to find any stages in such a process. Concerning their function Marshall was likewise unable to come to a definite conclusion, but suggests that they may act upon the nurse-cell substance making it available for the oocyte.

As described on page 509, the secondary nuclei of *Camponotus* make their appearance at Stage E (fig. 14, *E*; fig. 27) in the growth of the oocyte. From this stage on the size of the primary nucleus does not increase but actually decreases and the number of secondary nuclei becomes greater as the oocyte enlarges (compare figs. 28, 31 and 33). At first the oocyte nucleus always lies very close to the center of the anterior pole of the oocyte and the secondary nuclei form a single layer in contact with the opposite wall of the oocyte nucleus (figs. 27, 28, 29), but in later

stages (figs. 30, 33) the group of nuclei is more often near one side, at the anterior pole, and the oocyte nucleus is entirely surrounded by secondary nuclei, the latter sometimes being several layers in thickness (fig. 34). Hundreds of such groups were carefully examined, beginning with oocytes in Stage D (fig. 14, *D*; fig. 26), but in no case could the origin of the secondary nuclei be definitely determined. As the latter increase in number, they, as well as the oocyte nucleus, tend to lose their spherical shape and become oblong, or indented, or more or less irregular (figs. 28, 29, 34). This may be due to the action of the fixing solution, or to the pressure of one upon another, but many of them present shapes very suggestive of budding, or of more or less equal constriction into two. Some groups selected from the large number examined are shown in outline in figure 45. Frequently the space produced by the indentation of one of the nuclei is perfectly clear and resembles a vacuole. This suggests the possibility that the irregularity of the nucleus may be due to the escape of material from it which occupies the space formed by the caving in of the nuclear membrane. If this material were then to become surrounded by a nuclear membrane a secondary nucleus would be the result.

Two other theories have been suggested to account for the formation of secondary nuclei. According to Will ('84) in Hemiptera, Korschelt in *Bombus*, Henneguy in the honey-bee, and Brunelli ('04) in Hymenoptera they appear to come from follicular epithelial cells. Brunelli thinks they are attracted around the germinal vesicle by chemical action. Gross ('03) likewise believes from his studies on *Bombus* and other Hymenoptera that they are true nuclei, but that they originate from the epithelial cells which are situated among the nurse cells. This cannot of course be true in forms such as *Camponotus* where the secondary nuclei appear before a follicle and nurse cells are acquired. The other theory is that advanced by Stuhlmann who says "Ich wiederhole noch einmal, dass ich diese Kerne nur für 'Dotterconcretionen' halte."

The investigations of Loyez ('08) upon the 'noyaux de Blochmann' are the most thorough yet published. She studied these

secondary nuclei or 'pseudo-noyaux' as she calls them, in four species of *Bombus*, two species of *Vespa*, and one species of *Xylocopa*. They were found to resemble true nuclei in their fully developed condition, but all stages were observed between these and the very small vacuole-like bodies from which they apparently arise. The theories of their origin by budding off from the germinal vesicle and by the emigration of epithelial cells are considered by Loyez to be untenable. The conclusion is reached that they originate from the germinal vesicle, follicular epithelial cells, and nurse cells not by budding or the emigration of entire nuclei "mais résultent d'une coagulation de substances venues du dehors à l'état fluide ou granuleux et modifiées par le cytoplasme de l'oeuf." (p. 100). In old oocytes the secondary nuclei were found to change in structure so that they resemble nuclei which are undergoing synapsis, and, since all stages between the typical secondary nucleus and a homogeneous globule were observed, Loyez decides that they transform into deutoplasmic spheres.

The presence of these secondary nuclei in certain insects and not in others can be regarded as a sort of precocious diminution of nuclear substances. The loss of chromatin by passage through the nuclear membrane and its identification as chromidia in the cytoplasm has been reported by a number of investigators as taking place in the nuclei of many different kinds of cells during what is known as the resting stage. During ordinary mitosis only a part and sometimes the smaller part of the nuclear chromatin is concerned in the formation of the spireme, the rest being cast out into the cytoplasm with the other nuclear contents when the membrane breaks down. These substances become scattered and dissolved in the cytoplasm. Just before the maturation divisions occur, it is customary for the germinal vesicle to liberate into the cytoplasm a considerable part of its contents, including granules or small masses of chromatin which become scattered amid the yolk globules and disappear. The diminution of nuclear substance therefore seems to be a widespread process. That the formation of at least part of the secondary nuclei in the oocytes of certain insects is likewise a nuclear

diminution process also seems probable. Each secondary nucleus contains masses of chromatin and in every way resembles a true nucleus, but, as in other cases of diminution, this chromatin and the other contents of the secondary nuclei are lost in the general egg substance. The elimination of this material simply occurs in these species at an earlier stage than in the oocytes of other animals.

The function and fate of the secondary nuclei cannot be stated with any degree of certainty. We have seen that they cease to form a compact group in the older oocytes and become distributed throughout the egg, especially near the periphery (figs. 37 and 39). Later they undergo a process which appears to be degenerative, and, according to those who have studied later stages, finally disappear altogether. The writer suggested a few years ago (Hegner '09) that secondary nuclei of this sort might migrate to the posterior pole and take part in the formation of the germ-line-determinants, but thus far no actual evidence that this occurs has been obtained. Marshall ('07) has expressed the opinion that they may make the substances provided by the nurse cells available for the oocyte. It also is possible, as Loyez claims, that these secondary nuclei may have some function in the formation of yolk.

Summary of Part II. 1. The ovarioles of *Camponotus* consist of four distinct regions (fig. 13), (a) a terminal filament, (b) a terminal chamber, (c) a zone of growth free from bacteria-like rods, and (d) the posterior part in which the oocytes are arranged in a linear series, are accompanied by nurse cells, and are surrounded and later invaded by the bacteria-like bodies.

2. The bacteria-like rods occupy definite regions of the ovariole. They are absent entirely from the terminal filament, terminal chamber and first zone of growth. In the rest of the ovariole they occur everywhere except in the nurse cells (fig. 25). The oocyte is at first free from them (fig. 25) but later is invaded (fig. 26) and almost completely filled with them (fig. 29). The rods are arranged at first in bundles (figs. 25, 29), but later become scattered (figs. 35, 36). As the oocyte increases in size and

yolk formation proceeds, they gradually disappear until none are visible except near the periphery in the posterior region.

3. Secondary nuclei appear near the oocyte nucleus at an early stage of growth (fig. 27). They increase in number, finally completely surrounding the germinal vesicle (figs. 33, 34). They later become distributed throughout the oocyte especially near the follicular epithelium (figs. 37, 39). Their origin by budding from the oocyte nucleus, or by the immigration of epithelial cells seems improbable. The conclusion is reached that the oocyte nucleus gives off materials into the cytoplasm which become enclosed by a membrane and develop into nuclear-like bodies. The fate of the secondary nuclei was not determined.

III. HISTORY OF THE NUCLEI AND GERM-LINE DETERMINANTS IN THE OOCYTES OF CERTAIN PARASITIC HYMENOPTERA AND HYMENOPTEROUS GALL-FLIES

1. *Copidosoma gelechiae*

In June, 1914, I published a short account of the growth of the oocyte in *Copidosoma gelechiae* with special reference to the origin of the germ-line-determinants. Since then two other accounts have appeared on the same subject, one by Martin ('14) on *Ageniaspis* (*Encyrtus*) *fuscicollis*, and the other by Silvestri ('14) on *Copidosoma buyssoni*. I have also been able to obtain and study a new lot of material. This makes it possible for me to add to my previous account and to clear up certain points about which differences of opinion have arisen. These poly-embryonic Hymenoptera are interesting principally because of the peculiarities in their embryonic development. We shall refer in this paper to two of these; (1) the history of the egg nucleus and (2) the origin and fate of the germ-line-determinants.

Silvestri ('06-'08) has shown that a body which he considered the nucleolus of the germinal vesicle is present near the posterior end of the eggs of certain parasitic Hymenoptera. During embryonic development this body is segregated in a single cleavage cell until the seven-cell stage is reached; then, having disintegrated, its substance is divided between two cells. These,

according to Silvestri, are the parents of all of the germ cells, a conclusion that seems justified, since a similar body in monembryonic parasites has been definitely traced until it becomes distributed among the germ cells.

In my preliminary report on *Copidosoma gelechia* I pointed out the improbability of the origin of the 'nucleolo' of Silvestri from the nucleolus of the germinal vesicle, and concluded from the material I then possessed that this body consists of all of the chromatin from the oocyte nucleus which had formed into a compact mass. To explain the presence of both this body and an egg nucleus it was suggested that two oocytes might fuse end to end, the posterior one furnishing the 'nucleolo' and the other the nucleus. The eggs of these insects, when ready to be laid, are long, with a very slender bent portion between the two thicker ends, as shown in figure 54. My material consisted only of serial sections cut 2 and 4 μ thick, and, as Silvestri ('14) has pointed out, I considered sections through the anterior and posterior ends of an oocyte as sections of complete oocytes. This is a mistake that I now wish to acknowledge, but is one that could hardly be avoided without good *in toto* preparations. With the aid of such preparations I have been able to confirm Silvestri's account in most respects. My conclusion, however, that the 'nucleolo' of Silvestri is not the nucleolus of the oocyte nucleus is correct, and my account of the history of the oocyte nucleus up to its change into an oval mass of chromatin is also correct, as indicated by the study of new material, and by the confirmatory account by Martin in *Ageniaspis*. I am indebted to Dr. R. W. Glaser for this new material.

Martin ('14) records the presence of three kinds of cytoplasmic inclusions in the growing eggs of *Ageniaspis*: (1) a cloud of granules near the posterior end, (2) a 'nucleolus' also near the posterior end, and (3) a few chromatin granules cast out by the nucleus. The 'nucleolus' is of particular interest to us, since it is undoubtedly a body similar to the 'nucleolo' of Silvestri. Martin was able to trace this 'nucleolus' from the young oocytes to the three-cell stage in the cleavage of the developing eggs. It appears first as a small group of granules lying in the midst

of the cloud of granules mentioned above. It gradually increases in size, reaching its maximum dimensions about the time the egg reaches its full size. Then it becomes vacuolated and loses some of its affinity for stains. When the first cleavage division occurs, it passes entire into one of the two blastomeres. This blastomere does not divide as quickly as the other and a three-cell stage thus results, one cell containing the 'nucleolus' and the other two lacking this body. At this point the 'nucleolus' breaks down and can not be traced further. Regarding the origin of the 'nucleolus' Martin is not certain. He agrees with me that it is not derived from the nucleolus of the germinal vesicle, since, when it first appears, it is at the opposite end of the oocyte. Apparently it is built up by the aggregation of the deeply staining granules among which it lies, but where these granules originate was not determined.

The history of the oocyte nucleus. The ovaries of *Copidosoma* consist of a number of ovarioles, each of which contains a row of oocytes in various stages of growth, the oldest being situated near the posterior end. It is thus possible to find without much difficulty all stages in the growth period. We shall begin our account with an oocyte (Stage A, fig. 46) which has already acquired an epithelium and is accompanied by a nurse chamber. A close examination of such an oocyte (fig. 55) reveals a very large nucleus, containing an irregular, homogeneous mass of chromatin. A very thin layer of cytoplasm surrounds the nucleus.

The nucleus does not increase much in size during the growth period, but the oocyte enlarges rapidly because of the accumulation of cytoplasm. During the interval between Stages A and B (figs. 46, 47) both the oocyte and the oocyte nucleus become larger and oval. The chromatin now consists of what appears to be a long much coiled thread (fig. 56) and one is led to believe that the homogeneous mass in Stage A is really the same thread much more compactly coiled; in other words, in the condition as synezeisis. By the time Stage C (fig. 48) is reached the nucleus has again regained a spherical shape and the chromatic spireme has become spread out as shown in figure 57. Up to

this time the cytoplasm appears to be homogeneous throughout. Martin finds in *Ageniaspis* at this stage a cloud of granules in the posterior region (fig. 70) but nothing of the sort is present in my preparations, nor were such granules observed by Silvestri in *Copidosoma bussyoni*. Silvestri ('14), however, thinks he has discovered a group of granules at the posterior end of the nucleus at about this stage (fig. 69) which he suggests may lead to the formation of the oosoma (formerly called by him the 'nucleolo' and designated by me as a keimbahn or germ-line-determinant).

The nuclear phenomena are of considerable interest from this time on. The spireme becomes more and more open (Stages D, E, figs. 49, 50) and finally breaks up into thin, chromosomes of irregular shape (Stage F, fig. 51). These chromosomes then become shorter and thicker and appear to unite near their ends (Stage G, fig. 52). At first the pairs are scattered about within the nucleus (fig. 58) but they soon straighten out and become arranged in a parallel series with their points of union lying in the equator (Stage H, figs. 53, 59, 60). Spindle fibers could be seen, but apparently no centrosomes or asters are present. The number of pairs of chromosomes as indicated by cross sections of spindles of this sort seem to be twelve, the same number recorded by Silvestri for *C. bussyoni*, but several very clear sections contain only eleven (fig. 61).

Soon after the parallel arrangement of the chromosome pairs occurs, the egg reaches its full growth and attains its definite shape (Stage I, fig. 54). The mitotic figure then passes through the stages of condensation, as described in my preliminary report (Hegner '14 b). The chromosomes gradually get closer together and become shorter and thicker (fig. 62). Where their ends meet at the equator a ridge appears, which causes the complex to resemble a maltese cross (fig. 63). Soon the spaces between the chromosomes are entirely obliterated (fig. 64) and a homogeneous mass of chromatin results (fig. 65).

Silvestri has noted the parallel arrangement of these chromosome rods, but has evidently failed to observe their condensation. Martin, however, has reported a similar phenomenon in *Agenias-*

pis, although in this form the rods which condense seem to consist of single instead of double chromosomes (fig. 66). The history of the nucleus as recorded by Martin is as follows:

The chromatin in the very young oocyte is aggregated at the posterior side of the nucleus. As the oocyte grows, it spreads throughout the nucleus, forming numerous granules which are distributed upon a reticulum. Chromosomes are then formed and soon become arranged on a spindle, which becomes more and more compact until a single mass of chromatin results (fig. 67). This mass divides in polar body formation (fig. 68) apparently without the presence of spindle fibers or asters.

The germ-line-determinants in Copidosoma. The 'nucleolo' or germ-line-determinant appears in my material at about Stage D (fig. 49) at which time it is perfectly distinct, staining a deep black in iron hematoxylin. From this stage on it is invariably present, increasing in size until Stage F (fig. 51) is attained. Five methods of origin have been suggested for this body. (1) Silvestri's ('06) first idea that it consists of the nucleolus of the germinal vesicle was shown to be incorrect in my preliminary report (Hegner '14 b) and Silvestri has admitted his error (Silvestri '14). (2) My conclusion that it arises from the chromatin of the oocyte nucleus has on the other hand been disputed by Silvestri and I wish here to acknowledge the truth of his observations. (3) In his latest report Silvestri ('14) coins a new name for this body, calling it the 'oosoma,' and thinks that it may possibly arise from a heap of granules at the posterior end of the nucleus (fig. 69). (4) Martin accepts Silvestri's term 'nucleolus' for the body, but claims that in *Ageniaspis* it is gradually built up by the aggregation of granules which appear in the cytoplasm of the posterior region of the egg (fig. 70). (5) Since I have been unable to confirm with my material either of the methods of origin suggested by Silvestri and Martin and since this germ-line-determinant appears suddenly at about Stage D (fig. 49), I wish to propose another theory as to its genesis. In Part II of my series of "Studies on germ cells" (Hegner '14 a) I have expressed the following conclusion, after collecting and discussing all the literature on the origin and history of the germ-line-determinants in animals.

The most plausible conclusions from a consideration of these observations and experiments are that every one of the eggs in which Keimbahn-determinants have been described, consists essentially of a fundamental ground substance which determines the orientation; that the time of appearance of Keimbahn-determinants depends upon the precociousness of the egg; that the Keimbahn-determinants are the visible evidences of differentiation in the cytoplasm; and that these differentiated portions of the cytoplasm are definitely localized by cytoplasmic movements, especially at about the time of maturation.

This conclusion still seems to me the only tenable one at the present time and applies, I believe, to the germ-line-determinants in *Copidosoma*, as well as to those in other animals.

2. *Apanteles glomeratus*.³

Another Hymenopteron that resembles *Copidosoma* in some respects is *Apanteles*, a parasite of the larva of the cabbage butterfly. An abundance of material was obtained in the month of August, 1914. The pupae and recently emerged adults were liberated from the cocoons, and their abdomens were fixed either in Bouin's picro-formol solution or Carnoy's mixture. As in *Copidosoma*, the ovaries contain oocytes in all stages of growth and hence their history could be traced without much difficulty.

The history of the oocyte nucleus. Oocytes at a very early stage (fig. 71) acquire an epithelium (*e*) and are accompanied by a group of nurse cells (*n*). The chromatin is large in amount and massed into an irregular homogeneous body. As growth proceeds (fig. 72) this chromatin-mass expands, revealing the spireme of which it consists. Soon the entire nucleus is filled with a network of chromatin threads (figs. 73-76), a condition that persists for a considerable part of the growth period. When the oocyte has reached its definitive size (fig. 77), the chromatin threads contract into chromosomes which apparently unite in pairs, as in *Copidosoma* (fig. 60), and become arranged side by side upon an asterless spindle (fig. 78). This stage is followed by the condensation of the chromosomes, as shown in figure 79. No later stages in the nuclear history were present in my material,

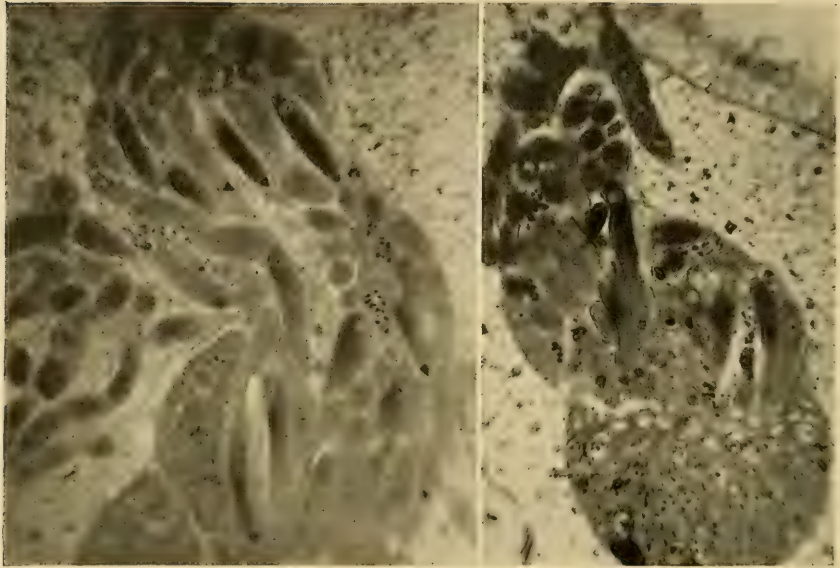
³ I am indebted to Dr. H. L. Viereck for the identification of this parasite.

but it is safe to assume that a further condensation occurs resulting in an oval, homogeneous mass as in *Copidosoma* (fig. 65).

The secondary nuclei When a stage about like that shown in figure 75 is reached, there appears within the cytoplasm of the anterior one-half of the oocyte a great number of spherical bodies which are arranged as in figure 75, and which resemble small nuclei. Figure 76 is an enlarged drawing of the anterior end of the section shown in figure 75. The secondary nuclei vary considerably in size. The substance within them stains like chromatin and is in the form of one or several small masses from which a few strands of chromatin granules radiate toward the membrane. These secondary nucleoli are present for only a brief period, having all disappeared by the time the chromosomes are formed (fig. 77). Their origin and function are problematical, but it seems hardly possible that they can arise from the germinal vesicle by budding, and hence we are forced to the same conclusions already stated in the case of *Camponotus* (p. 371).

The germ-line-determinants. The fully grown oocytes of *Apanteles* contain the most conspicuous germ-line-determinants yet described (fig. A). Although its history during embryonic development is not known, the probability that it plays an important rôle in the formation of the primordial germ cells is so great that it seems safe to include it among the bodies to which the term *keimbahn* or germ-line-determinant has been applied.

The first indication of this body occurs in a half-grown oocyte (fig. 73). Here a triangular area at the extreme posterior end may be distinguished from the rest of the egg by a slightly greater staining capacity (somewhat exaggerated in fig. 73). This affinity for basic stains increases as the oocytes grow older, and a network appears (fig. 74) which very much resembles the 'netzapparat' described by many writers both in germ cells and somatic cells (Duesberg '11). In succeeding stages this network condenses into a solid mass (fig. 75), but cavities soon appear again (figs. 77, 80), and the threads become thinner (fig. 81). Finally a condition is reached (fig. 82) in which the threads break



Text fig. A Microphotographs of longitudinal sections through the abdomen of *Apanteles* showing various stages in the growth of the oocytes. The germ-line determinants appear as distinct triangular black bodies near the posterior ends of certain of the larger oocytes.

up into a large number of irregular masses, suspended in a homogeneous substance. Near the germ-line-determinant in later stages (fig. 75) are a number of large widely scattered granules which are probably separated off from the main body.

The resemblance between this body and the pole-plasm in the egg of *Miastor* (Kahle '08; Hegner '12, '14 a, '14 c) is quite striking. The pole-plasm in *Miastor* appears just before the oocyte undergoes maturation, and apparently does not arise directly from the germinal vesicle, nurse cells, or follicular epithelium, but is a visibly differentiated portion of the cytoplasm that has become localized at the posterior end. What causes this differentiation is not known, but a discussion of the subject will be found in my previous contributions (Hegner '14 a, '14 c). In *Miastor* the pole-plasm never proceeds beyond the granular stage, but in *Apanteles* a rather definite series of conditions

ensues during which the granular stage (fig. 73) is succeeded by a heavy network (fig. 74); this is followed by condensation into a solid mass (fig. 75), the formation of a heavy network again (fig. 80), the thinning out of this network (fig. 81), and finally the breaking up of the threads into many large irregular granules (fig. 82).

3. *Hymenopterous gall-flies*

There is still much to be learned regarding the life-cycles of the gall-flies, but what we do know is enough to prove that these insects offer a very profitable field for research. Examinations of the growing oocytes of certain Hymenopterous gall-flies have revealed many interesting structures that have a bearing upon the conditions described in the preceding part of this contribution and, although much more work needs to be done, the data already obtained are included here to indicate the widespread occurrence of phenomena described above. The oogenesis in these insects is more difficult to study than in the parasitic Hymenoptera because fewer stages of growth are represented by the oocytes in a single individual.

The maturation spindle in the oak-knot gall-fly, Andricus punctatus. The oak-knot gall-fly lays a pear-shaped egg (fig. 83), from the anterior end of which extends a long, slender process with an expanded terminal portion. This process resembles those described by Korschelt ('87) in *Ranatra linearis*. The two long slender processes extending from the anterior end of the eggs of *Ranatra*, arise from a single bud-like protuberance at one side of the anterior end of the oocyte, and their place of origin alternates from one side to the other in the row of oocytes which lie in the lower part of the ovariole. When the eggs are laid, the processes are left extending freely out into the water from the decaying wood in which the rest of the egg is imbedded by the female.

The egg of the oak-knot gall-fly shown in figure 83, was taken from an adult which was just about to emerge from the gall. At one side near the anterior end could be seen a spindle-shaped body—the nucleus of the egg. Several stages in the develop-

ment of this body were found and they seem to indicate a condition similar to that described in *Copidosoma* and *Apanteles*. The earliest stage discovered (fig. 84) represents an asterless spindle bearing a number of pairs of chromosomes attached near their ends and drawn out so as to form a more or less parallel series. These pairs then condense, as shown in figures 85 and 86, and finally produce the pear-shaped body mentioned above (fig. 83). Apparently the chromosomes become completely fused in forming this body, since a high magnification (fig. 87) reveals nothing more than a vacuolated mass of chromatin. The nucleus in *Copidosoma* never seems to undergo vacuolization, nor does the similar body described in *Ageniaspis* by Martin ('14).

No body was found near the posterior end of the oocytes of the oak-knot gall-fly such as occur in those of *Copidosoma*, *Apanteles*, and the blackberry-knot gall-fly next to be described.

The maturation spindle and germ-line-determinants in the blackberry-knot gall-fly, Diastrophus nebulosus. The eggs of the blackberry-knot gall-fly (fig. 88) resemble in general shape and size those of the oak-knot gall-fly (fig. 83) and the nucleus is in a similar position. This nucleus forms a rather compact body, but not a homogeneous mass. The stage represented in figures 89, 90 and 91 may be one of a series ending in the production of such a mass, but no other stages were found. Figures 89 and 90 were drawn from longitudinal sections and show that the position of the oval nucleus may vary; figure 91 is from a transverse section.

At the posterior end of the egg (fig. 88) is a more or less spherical body to which we are justified, I believe, in applying the name, germ-line-determinant. This body stains black with hematoxylin and is filled with vacuoles (fig. 92). Weismann ('82) described a body near the posterior end of the eggs of *Rhodites rosae* which he called the 'Furchungskern,' but it is evident from his account and figures that this body is similar to the one I have just described and is not a cleavage nucleus. According to Weismann this body spreads out during cleavage and occupies

a large part of the posterior region; at this stage the term 'hinterer Polkern' is applied to it. Its later history was not followed.

It is worth mentioning that the follicle cells of the oocytes divide by mitosis (fig. 93) and not by amitosis as has been described in some insects.

Secondary nuclei in the oocytes of the mealy rose gall-fly Rhodites ignota. The eggs of this gall-fly (fig. 94) possess a very long anterior process, as in the two species already described, and the nucleus is similarly placed, but no body occurs at the posterior end. Of particular interest here is the presence of a large number of secondary nuclei at certain stages in the growth of the oocyte. These secondary nuclei were first observed near the periphery, as indicated in (fig. 95), which is part of a transverse section. They are very small and appear to consist of a single body that stains like chromatin, and are surrounded by a membrane. The occurrence of deeply staining granules without these membranes, and the various sizes of the secondary nuclei formed, lead to the conclusion that chromatin granules from the oocyte nucleus, from the nurse cells, or from the follicle cells, migrate into the cytoplasm and become the center of origin of the secondary nuclei. In later stages these nuclei are all larger and form a layer a slight distance from the periphery of the oocyte (fig. 96). They vary greatly in size as shown in figure 97, but exhibit all the characteristics of true nuclei. No secondary nuclei could be found in older oocytes, but what becomes of them was not determined.

*Summary of Part III.*⁴ *A. Copidosoma gelechiae.* 1. The chromatin in the oocyte nucleus forms chromosomes at an early stage in the growth period (fig. 51). These chromosomes unite near their ends in pairs (figs. 52 and 58) and then become arranged in a parallel series upon an asterless spindle (figs. 53, 59, 60). Condensation then occurs and an apparently homogeneous oval-shaped mass of chromatin is formed (figs. 54 and 62-65). The number of pairs of chromosomes is eleven (fig. 61) or twelve. The nuclear history is essentially as described in my preliminary

⁴ Summaries of Parts I and II will be found on pages 505 and 520.

report (Hegner '14 b) and similar to that described by Martin ('14) in *Agenaspis*.

2. The germ-line-determinant is not the chromatin from an oocyte nucleus, as stated in my preliminary paper, but it appears to be a differentiated part of the protoplasm which arises at an early stage (fig. 49) near the posterior end of the oocyte.

B. Apanteles. 1. The oocyte nucleus has a history similar to that described for *Copidosoma*. Chromosomes are formed at an early period, fuse in pairs, become arranged upon an asterless spindle (figs. 77-78), and undergo condensation (fig. 79). Whether or not they finally form a homogeneous mass could not be determined because of the lack of late stages.

2. Secondary nuclei make their appearance in the almost fully grown oocytes. They are distributed throughout the anterior half of the oocyte (figs. 75-76), but are entirely absent in later stages (fig. 77). Their origin and fate were not determined.

3. The deeply staining substance at the posterior end of the older oocytes is probably a germ-line-determinant. It first appears in a partially grown oocyte as a dark granular mass, which probably represents a differentiated part of the protoplasm (fig. 73). Later it passes through the stages described on pages 527-529 and illustrated in figures 74, 75 and 80 to 82.

C. Gall-flies. 1. The history of the oocyte nucleus of the oak-knot gall-fly resembles very closely that of *Copidosoma* and *Apanteles* (figs. 84-87).

2. The oocytes of the blackberry-knot gall-fly contain a chromatin body (figs. 88-91) which probably results from the condensation of chromosomes as in the other forms described above. A conspicuous germ-line-determinant is also present near the posterior end (figs. 88, 92); the follicle cells divide by mitosis (fig. 93).

3. The half-grown oocytes of the mealy rose gall-fly are provided with hundreds of secondary nuclei (fig. 97) which are situated in a single layer equidistant from the periphery at all points (fig. 96). In younger oocytes (fig. 95) these secondary

nuclei appear to arise near the periphery from granules which stain like chromatin. These granules may be extruded by the oocyte nucleus, the follicle cells or the nurse cells.

February 19, 1915

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PLATE 1

EXPLANATION OF FIGURES

Apis mellifica

1 Outline of an ovariole showing the terminal filament, *t*, a zone containing rosettes of cells, *r*, a zone of differentiation, *d*, a region containing a linear series of oocytes, *o*, and nurse cells, *n*, accompanying an oocyte. $\times 110$.

2 Part of the rosette region of an ovariole; *r*, a rosette, the cells of which are held together by deeply staining strands; *e*, an epithelial cell nucleus. $\times 650$.

3 A single rosette in longitudinal section. $\times 1900$.

4 A single rosette in transverse section. $\times 1900$.

5 Synchronous division of the cells in a rosette. $\times 1900$.

6 Part of the zone of differentiation of an ovariole; *o*, oocyte; *e*, epithelial cell nuclei; *n*, nurse cell. $\times 430$.

7 Two neighboring oocytes from the zone of differentiation showing the double rings connecting them with each other and with surrounding nurse cells. $\times 1900$.

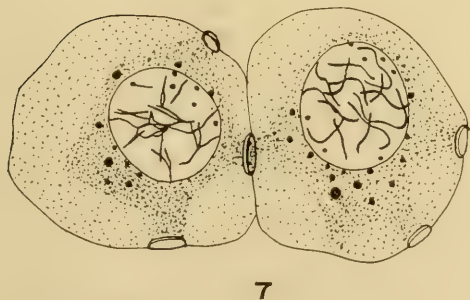
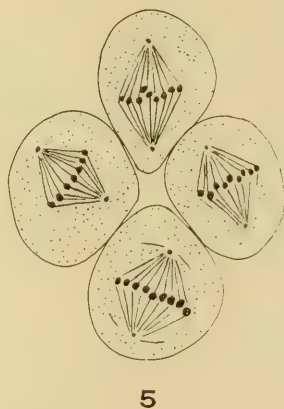
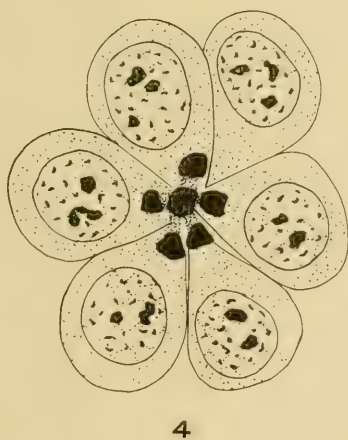
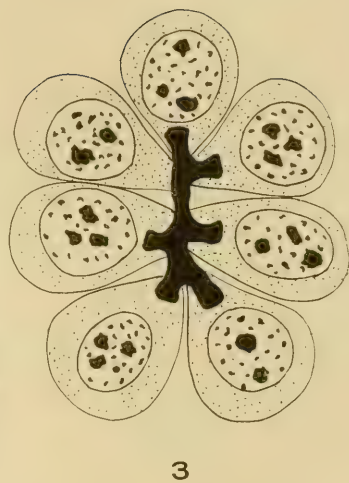
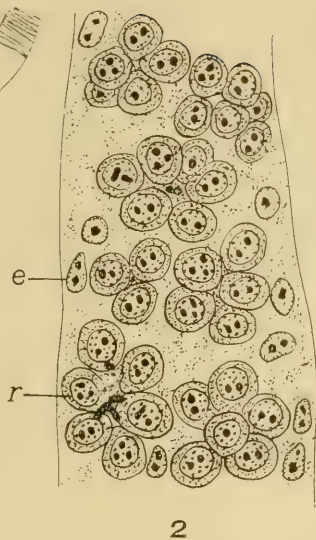
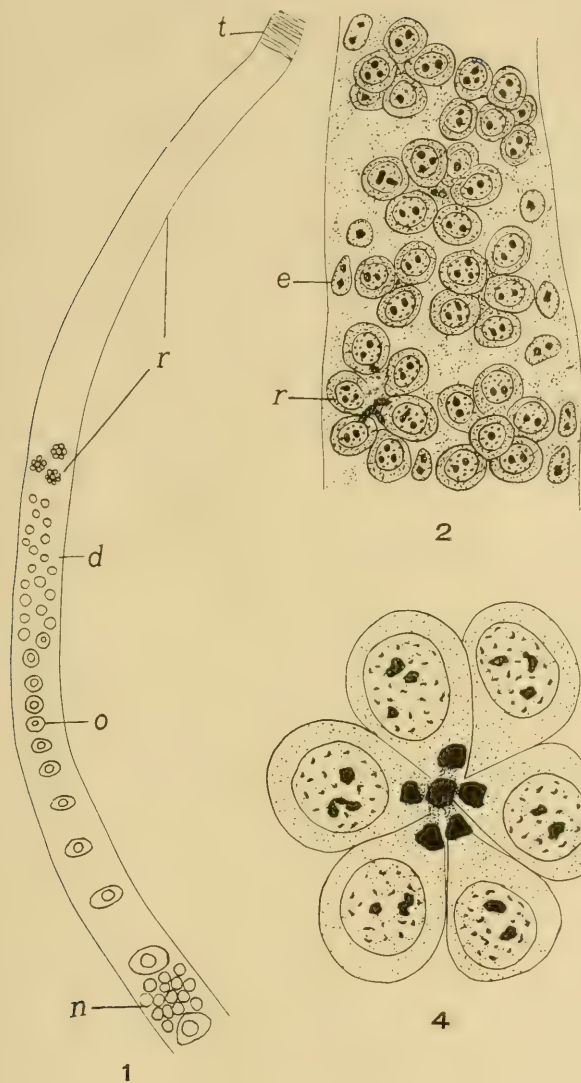
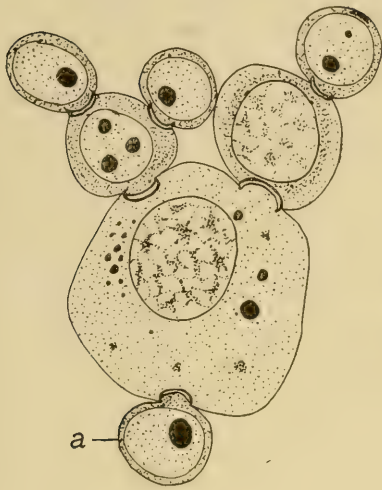


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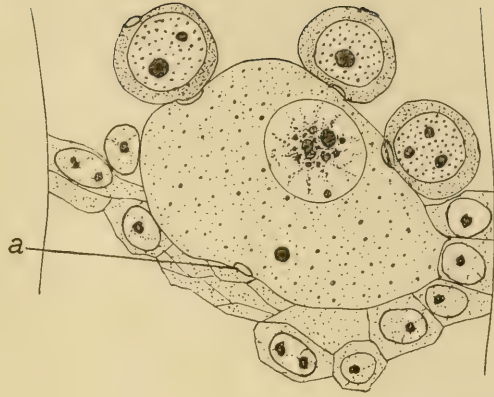
EXPLANATION OF FIGURES

Apis mellifica

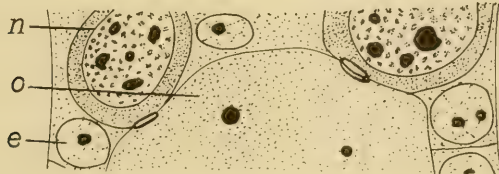
- 8 A group of nurse cells surrounding an oocyte. $\times 1900$.
- 9 An older oocyte with nurse cells and epithelial cells. $\times 1250$.
- 10 An outline showing the arrangement of an oocyte and its accompanying nurse cells. $\times 1250$.
- 11 Part of a rather old oocyte, *o*, still connected with nurse cells, *n*, by means of rings, *e*, epithelial cell. $\times 1250$.
- 12 An outline of an older oocyte showing the rings between the nurse cells and oocyte and between neighboring nurse cells. $\times 430$.



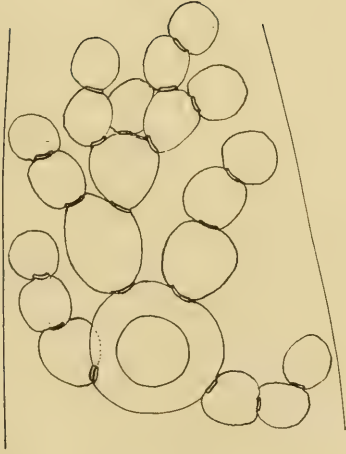
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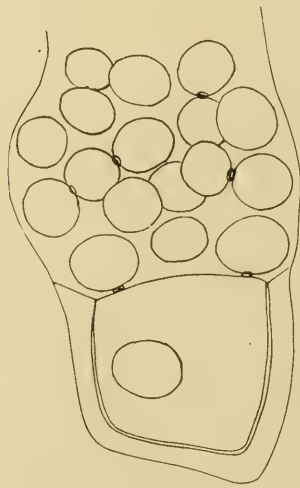
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12

PLATE 3

EXPLANATION OF FIGURES

Camponotus herculeanus var. *pennsylvanica*

13 Outline of an ovariole showing the terminal filament, *t*, terminal chamber, *tc.*, first zone of growth, *g*, and later growth zone containing oocytes, *o*, and nurse cells, *n* and *nc.* × 170.

14 Outlines of oocytes in Stages A to G. × 110.

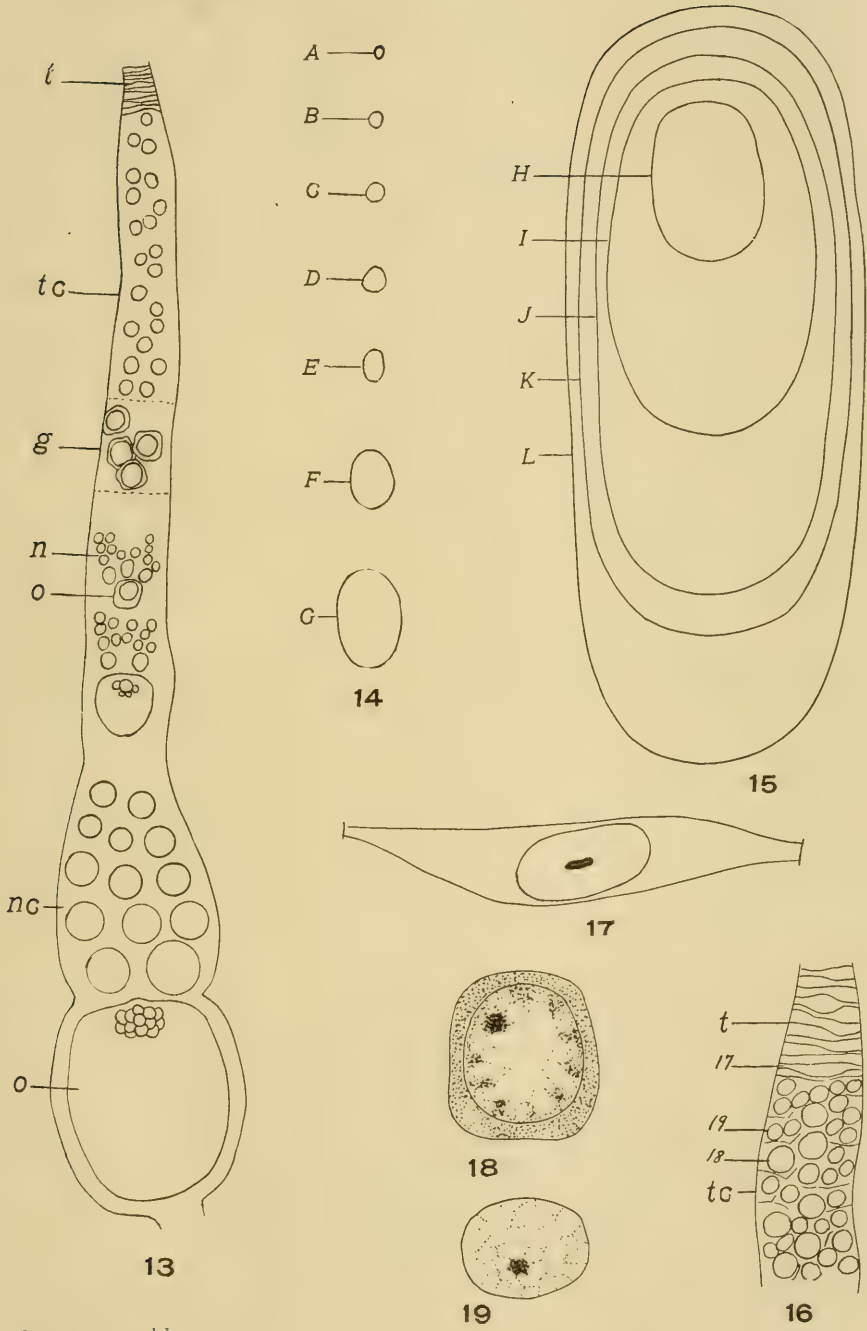
15 Outlines of oocytes in Stages H to L. × 110.

16 Outline of part of the terminal filament, *t*, and terminal chamber, *tc.* The numbers 17, 18 and 19 refer to cells shown enlarged in figures 17, 18 and 19. × 620.

17 A single cell from the terminal filament. × 3300.

18 An oocyte from the terminal chamber. × 3300.

19 A nurse cell nucleus from the terminal chamber. × 3300.



R. W. HEGNER, *del.*

PLATE 4

EXPLANATION OF FIGURES

Campanotus herculeanus var. *pennsylvanica*

20 Outline of the first zone of differentiation, showing the membrane, *m*, separating it from the terminal chamber, and the oocytes, *o*, nurse cells, *n*, and epithelial cells, *e*. The numbers 21, 22 and 23 refer to cells shown enlarged in figures 21, 22 and 23. $\times 620$.

21 An oocyte from the first zone of growth. $\times 3300$.

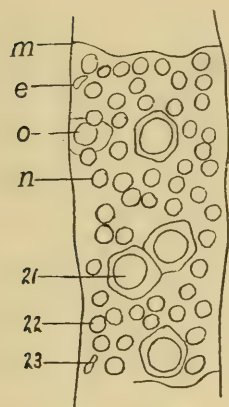
22 A nurse cell from the first zone of growth. $\times 3300$.

23 An epithelial cell nucleus from the first zone of growth. $\times 3300$.

24 The posterior portion of the first zone of growth and the anterior portion of the rest of the ovariole containing bacteria-like rods. *e*, epithelial cell nucleus; *n*, nurse cell; *o*, oocyte. $\times 1250$.

25 Part of an ovariole showing two oocytes, C_1 and C_2 , in Stage C. *n*, nurse cell. $\times 1250$.

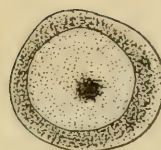
26 An oocyte in Stage D. The bacteria-like rods have invaded the cytoplasm of the oocyte. $\times 1250$.



20



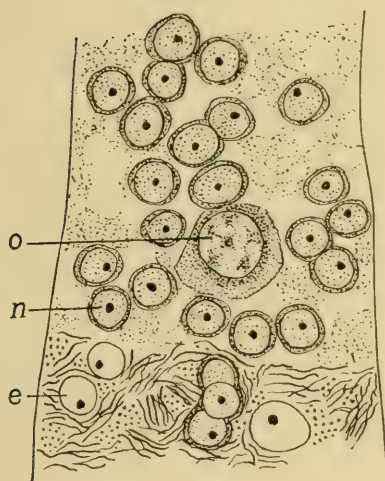
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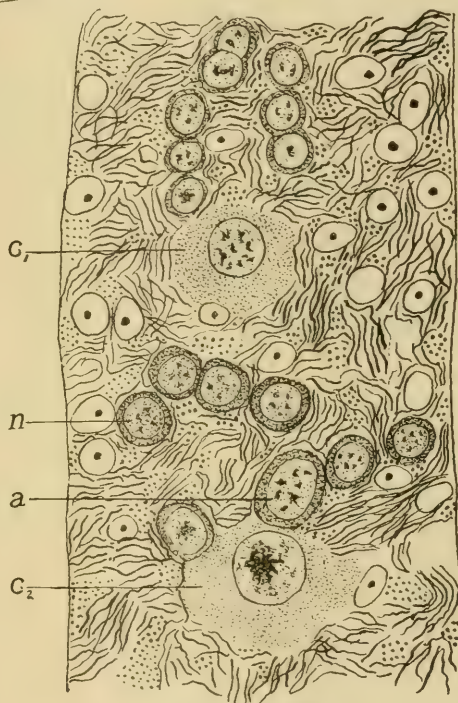
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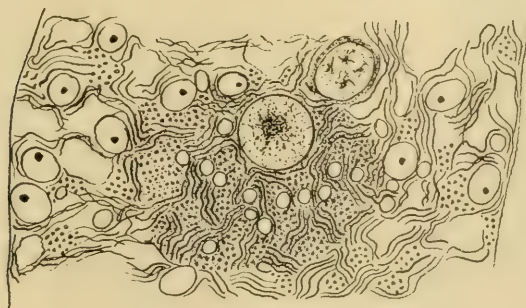
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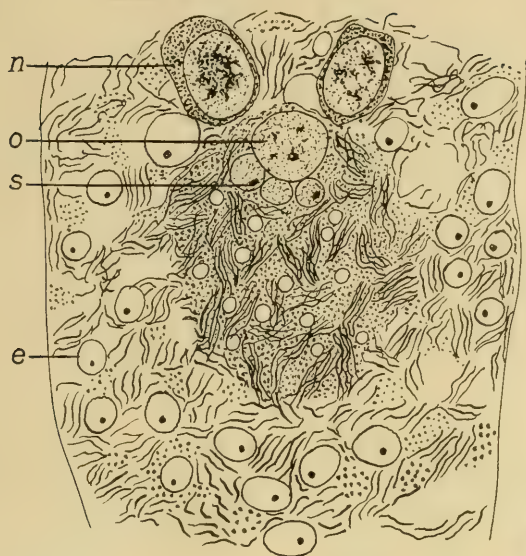
R. W. HEGNER, *del.*

PLATE 5

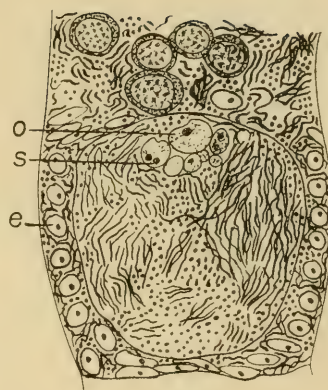
EXPLANATION OF FIGURES

Camponotus hereculeanus var. *pennsylvanica*

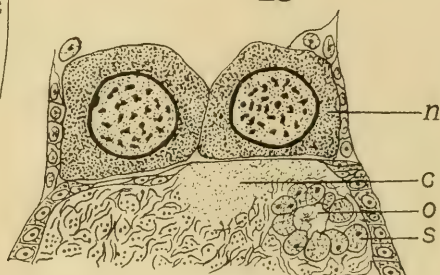
- 27 An oocyte in Stage E showing three secondary nuclei, *s*, near the oocyte nucleus, *o*. *e*, epithelial cell nucleus; *n*, nurse cell. $\times 1250$.
- 28 An oocyte in Stage F. Lettering as in figure 27. $\times 620$.
- 29 An oocyte in Stage G. Lettering as in figure 27. $\times 620$.
- 30 Part of an oocyte and two nurse cells, *n*. Cytoplasm, *c*, elaborated by the nurse cells is present near the nurse chamber. *o*, oocyte nucleus; *s*, secondary nuclei. $\times 430$.
- 31 Transverse section through the anterior end of an oocyte. $\times 620$.
- 32 Part of the nucleus of a nurse cell showing vacuoles and deeply staining granules in the thick nuclear membrane. $\times 3300$.



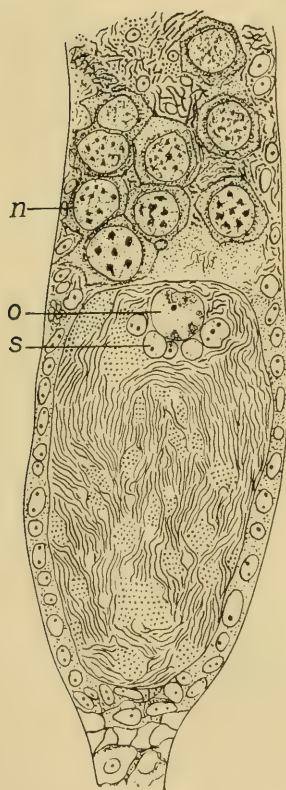
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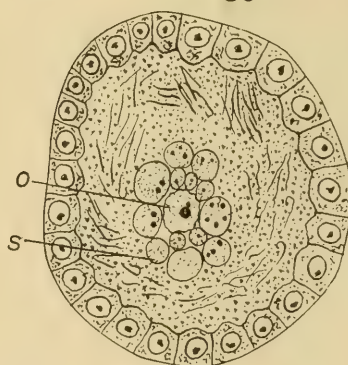
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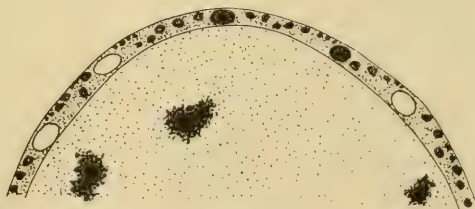
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PLATE 6

EXPLANATION OF FIGURES

Camponotus hereculeanus var. *pennsylvanica*

33 Part of an oocyte in Stage H showing its connection, *a*, with the nurse chamber; *o*, oocyte nucleus; *s*, secondary nuclei. $\times 430$.

34 An oocyte nucleus surrounded by secondary nuclei from an oocyte in Stage H. $\times 1250$.

35 Part of an oocyte in Stage I. *e*, follicular epithelium; *k*, 'Keimhautblastem'; *y*, yolk globules. $\times 430$.

36 Part of an oocyte in Stage J. $\times 430$.

37 The anterior part of an oocyte showing the breaking up of the group of secondary nuclei, *s*. *a*, connection with nurse chamber. *c*, cytoplasm; *e*, follicular epithelium; *y*, yolk globules. $\times 430$.

38 A single secondary nucleus and three yolk globules in Stage K. $\times 1900$.

39 Part of the edge of an oocyte in Stage L showing the follicular epithelium and the distribution of secondary nuclei and yolk globules. $\times 430$.

40 Two secondary nuclei and two yolk globules, enlarged, from an oocyte in Stage L. $\times 1900$.

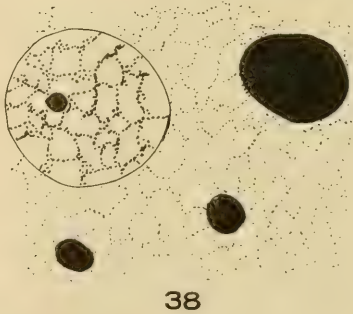
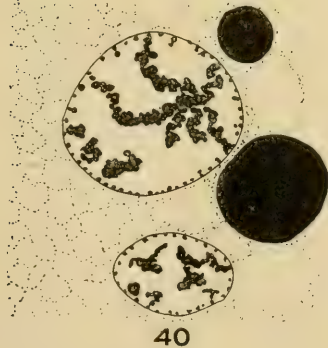
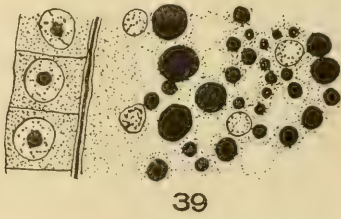
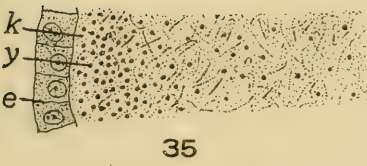
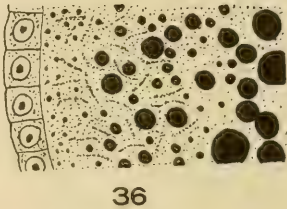
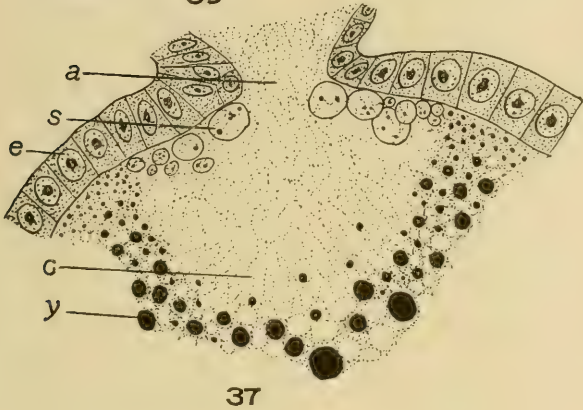
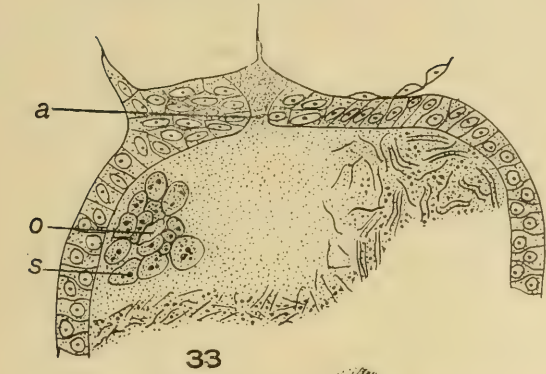


PLATE 7

EXPLANATION OF FIGURES

- 41 Longitudinal section through an egg of *C. herculeanus* var. *ferrugineus* one hour old. $\times 52$ (from Tanquary).
- 42 Ditto, twenty hours old. $\times 52$ (from Tanquary).
- 43 Ditto, slightly older. $\times 52$ (from Tanquary).
- 44 Ditto, two days old. $\times 52$ (from Tanquary).
- 45 Outlines of oocyte nuclei (dotted in) with their accompanying secondary nuclei of *C. herculeanus* var. *pennsylvanica*. $\times 1250$.

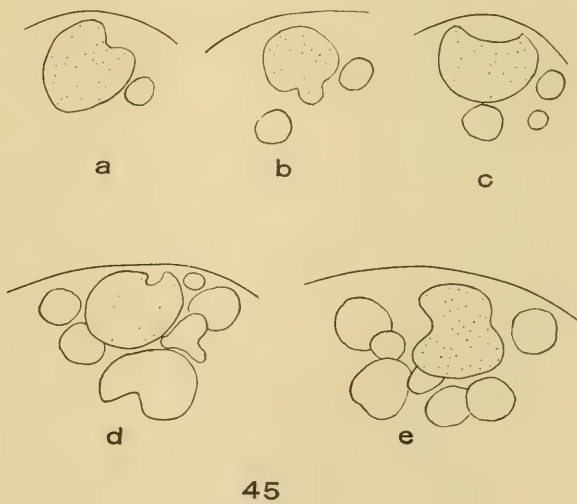
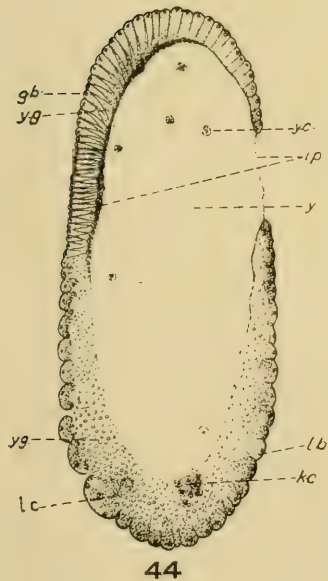
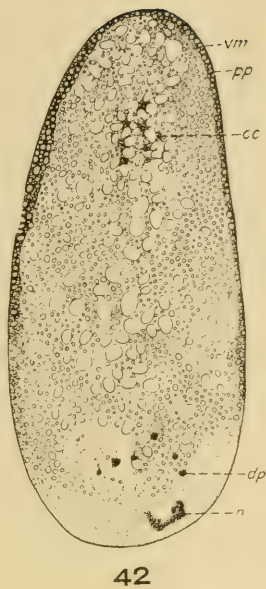
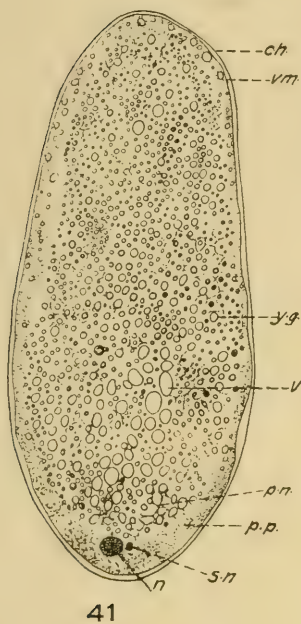


PLATE 8

EXPLANATION OF FIGURES

Copidosoma gelechia

- 46 Outline of a young oocyte in Stage A surrounded by a follicular epithelium and accompanied by a group of nurse cells. $\times 1250$.
- 47 Outline of an oocyte in Stage B. The follicular epithelium is shown, but the nurse cells have been omitted. $\times 1250$.
- 48 Outline of an oocyte in Stage C. $\times 1250$.
- 49 Outline of an oocyte in Stage D. First appearance of the germ-line-determinant near the posterior end. $\times 1250$.
- 50 Outline of an oocyte in Stage E. $\times 1250$.
- 51 Outline of an oocyte in Stage F. Single chromosomes are present. $\times 1250$.
- 52 Outline of an oocyte in Stage G. The chromosomes have united near their ends to form pairs. $\times 1250$.
- 53 Outline of an oocyte in Stage H. The pairs of chromosomes are arranged in a parallel series. $\times 1250$.
- 54 Outline of an oocyte in Stage I. $\times 1250$.

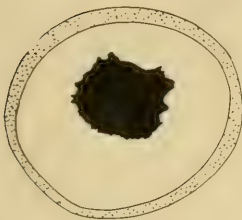


PLATE 9

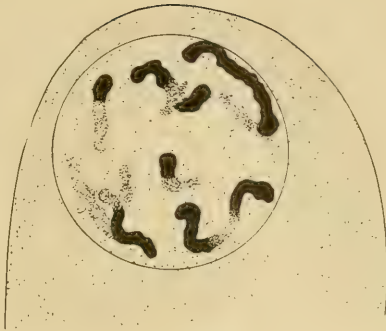
EXPLANATION OF FIGURES

Copidosoma gelechia

- 55 An oocyte in Stage A (see fig. 46). $\times 3300$.
- 56 An oocyte in Stage B (see fig. 47). $\times 3300$.
- 57 An oocyte in Stage C (see fig. 48). $\times 3300$.
- 58 The anterior portion of an oocyte in Stage G (see fig. 52). $\times 3300$.
- 59. The nucleus of an oocyte in Stage H (see fig. 53). $\times 3300$.
- 60 The nucleus of a slightly older oocyte. $\times 3300$.
- 61 A transverse section through a nucleus in a similar condition. $\times 3300$.
- 62 to 65 Successive stages in the condensation of a spindle like that shown in figure 60. $\times 800$.



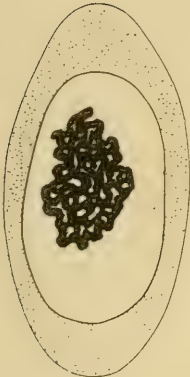
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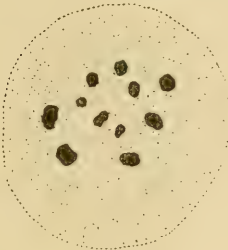
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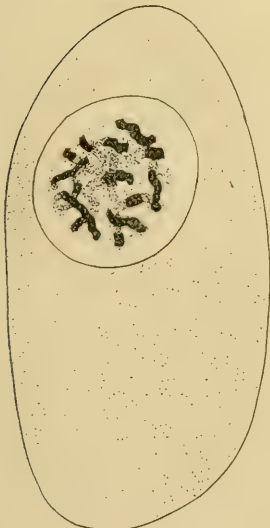
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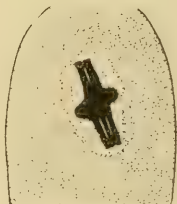
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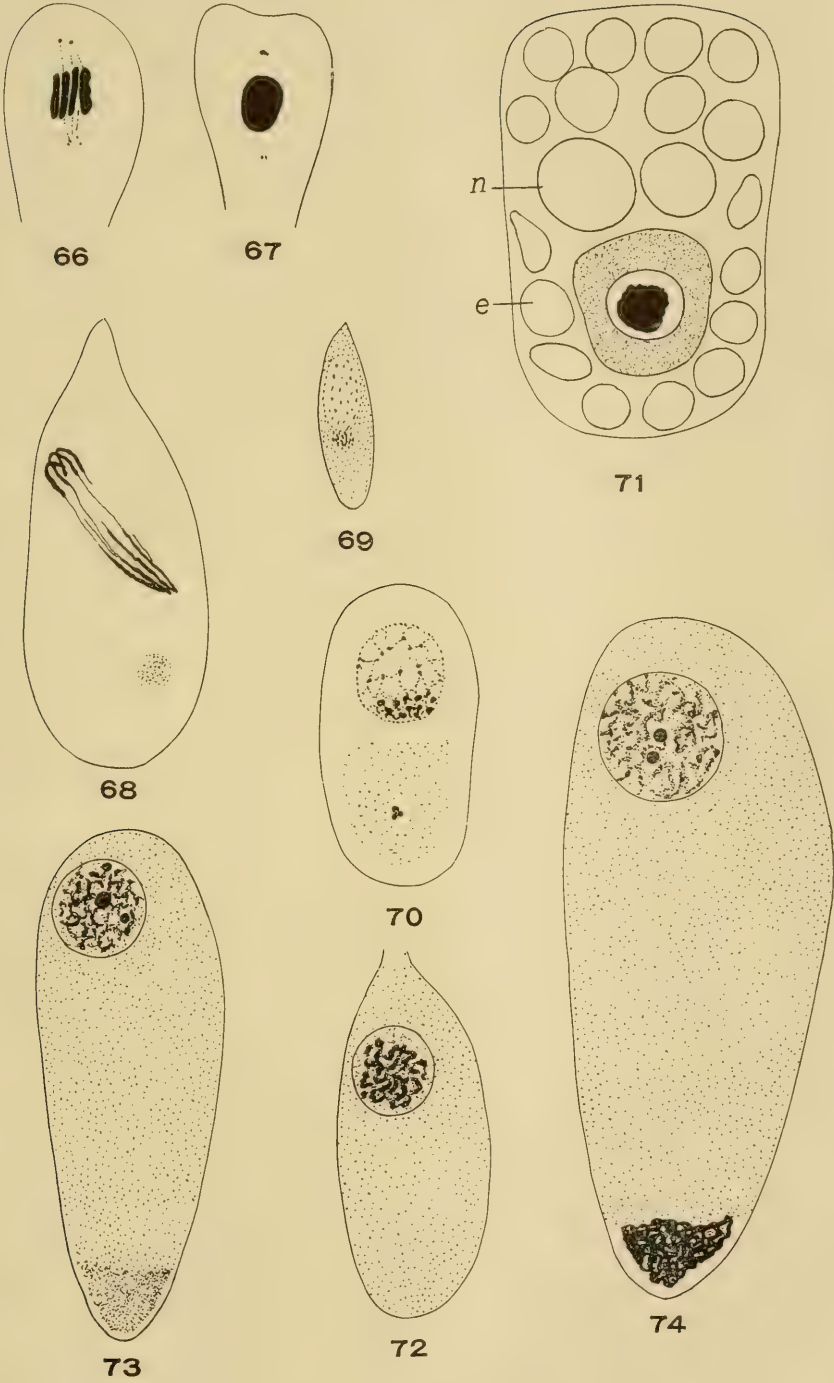


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PLATE 10

EXPLANATION OF FIGURES

- 66 Ageniaspis; the anterior portion of an oocyte showing the arrangement of the chromosomes on the spindle (after Martin).
- 67 Ageniaspis; a later stage showing the mass of chromatin resulting from the condensation of the chromosomes (after Martin).
- 68 Ageniaspis; the first maturation division of the egg (after Martin).
- 69 Copidosoma; a young oocyte showing a group of granules near the posterior end of the nucleus (after Silvestri).
- 70 Ageniaspis; a young oocyte containing a cloud of granules in the posterior portion and a larger body, the 'nucleolus' (after Martin).
- 71-74 Apanteles.
- 71 A young oocyte surrounded by epithelial cells, *e*, and accompanied by nurse cells, *n*. $\times 1900$.
- 72 An older oocyte. $\times 1900$.
- 73 An older oocyte showing the first appearance of the germ-line-determinant. $\times 1900$.
- 74 A still older oocyte. $\times 1900$.



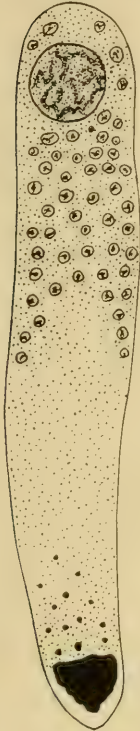
R. W. HEGNER, *del.*

PLATE 11

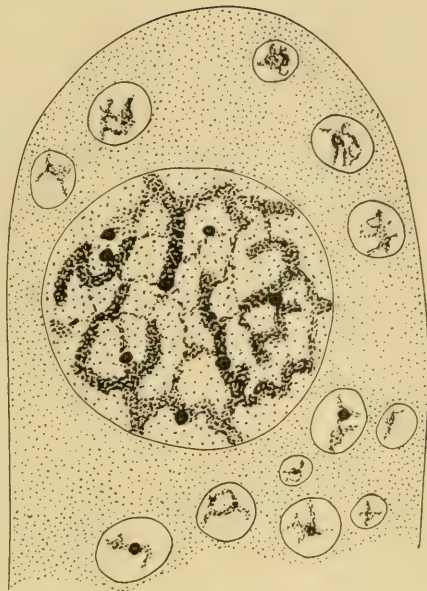
EXPLANATION OF FIGURES

Apanteles

- 75 An oocyte containing many secondary nuclei. $\times 850$.
- 76 Part of the oocyte shown in figure 75. $\times 3300$.
- 77 An older oocyte showing the parallel arrangement of chromosomes. $\times 850$.
- 78 Nucleus enlarged from figure 77. $\times 3300$.
- 79 A later stage in the history of the nucleus. $\times 3300$.
- 80-82 Successive stages in the history of the germ-line-determinant. $\times 1900$.



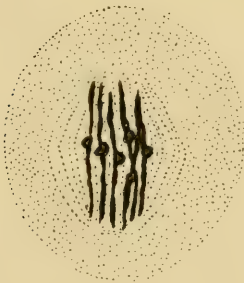
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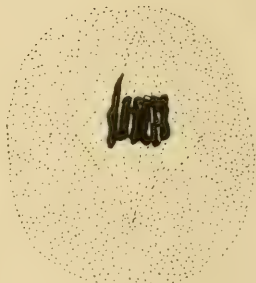
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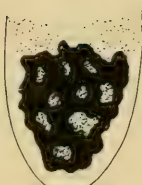
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PLATE 12

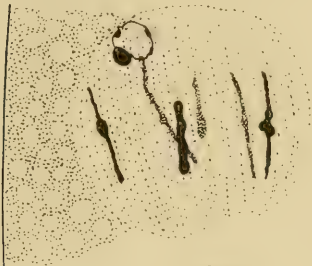
EXPLANATION OF FIGURES

Andricus punctatus

83 An egg ready to be laid. $\times 430$.

84-86 Nuclei showing stages in the condensation of the chromosomes.
 $\times 1900$.

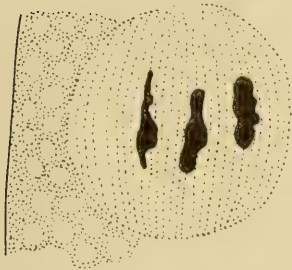
87 The chromatin mass resulting from the condensation of the chromosomes.
 $\times 1900$.



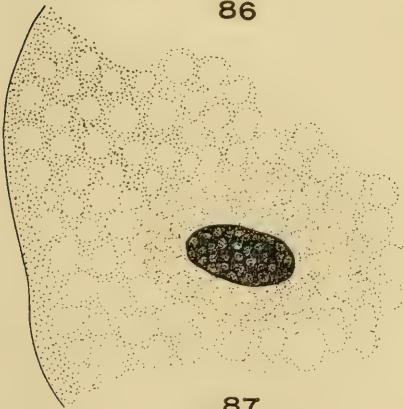
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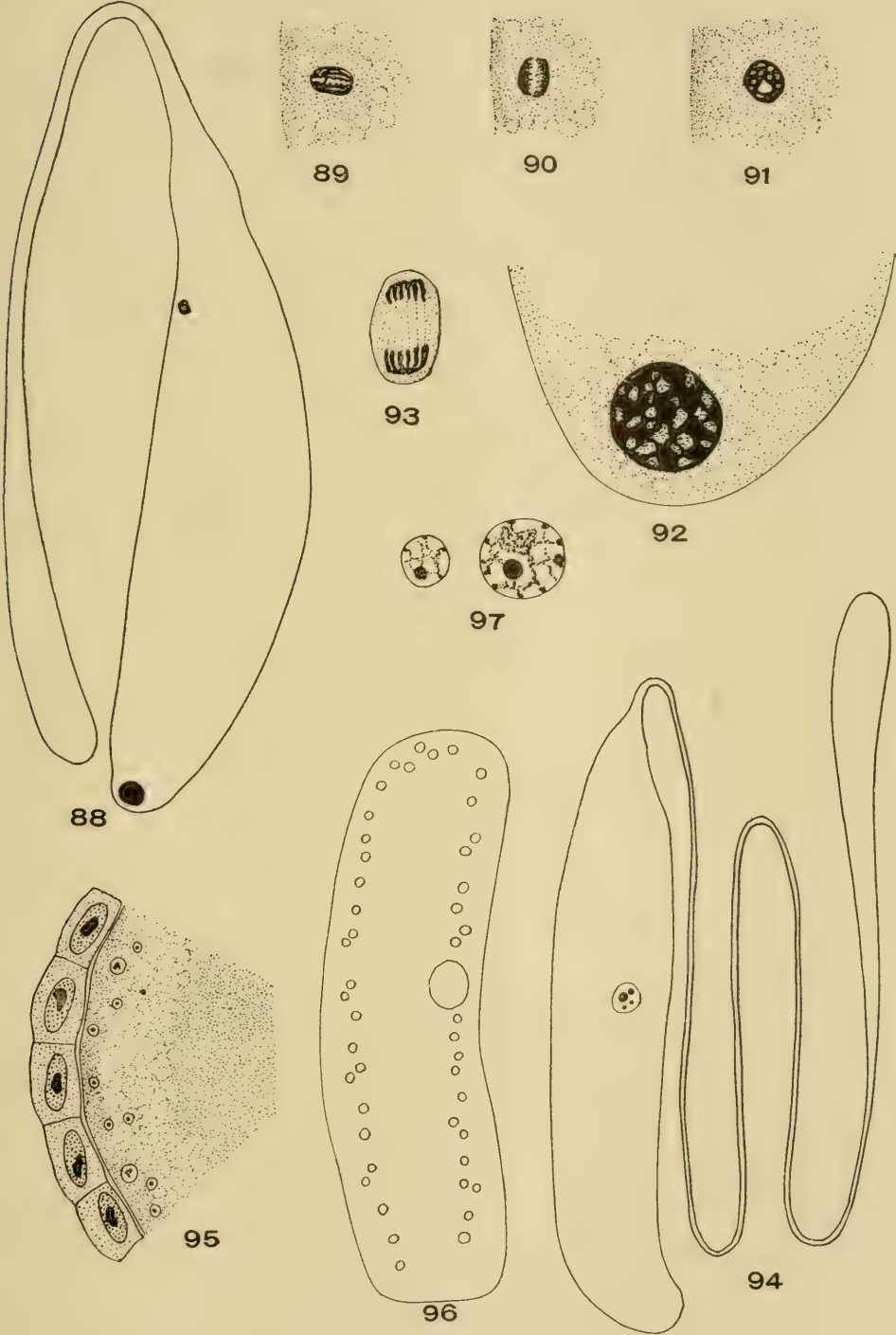


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PLATE 13

EXPLANATION OF FIGURES

- 88-93 *Diastrophus nebulosus*.
88 An egg ready to be laid. $\times 430$.
89-90 Longitudinal sections through the nucleus of such an egg. $\times 1900$.
91 Transverse section through a nucleus in the same stage. $\times 1900$.
92 The germ-line-determinant near the posterior end. $\times 1900$.
93 The mitotic division of a follicular epithelial cell. $\times 1900$.
94-97 *Rhodites ignota*.
94 An egg ready to be laid. $\times 430$.
95 Part of an oocyte showing stages in the formation of secondary nuclei.
 $\times 1900$.
96 An older oocyte showing the arrangement of secondary nuclei. $\times 620$.
97 Two secondary nuclei much enlarged. $\times 2500$.



THE HOMOLOGIES OF THE HYOMANDIBULA OF THE GNATHOSTOME FISHES

EDWARD PHELPS ALLIS, JR.

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ONE FIGURE

Work that I have under way on the cranial anatomy of *Chlamydoselachus* has led to certain conclusions regarding the hyomandibula which, if correct, are of considerable morphological importance. They are based on the assumption, which seems practically established by my work, that the dorsal ends of all of the so-called inner cartilaginous bars of all of the visceral arches, in all of the gnathostome fishes, always lie ventral to the vena jugularis, and that when parts of the cartilaginous bars of the adult fish articulate or fuse with the neurocranium dorsal to that vein, those parts are derived either from the external cartilaginous bars of the arches, or from interarcual cartilages developed in, or in relation to, the dorsal interarcual ligaments.

The terms inner and external cartilaginous bars, or arches, are here used with the significance commonly given to them, but my work tends decidedly to confirm Dohrn's conclusion that the so-called inner cartilaginous bars of the branchial arches of the gnathostome fishes, and not the external ones, are the homologues of the cartilaginous branchial arches of the Cyclostomata.

The cartilages which form the so-called external arches of the gnathostome fishes are commonly called the extrabranchials in whatever arch they may be found. Parker ('76) limited the use of this term to the external cartilages of the gill-bearing, branchial and hyal arches, employing the term extraviscerals to designate, collectively, these cartilages and certain others, the so-called labial cartilages of his descriptions, which he considered to be their serial homologues in the prehyal arches. The use of the term

extravisceral, thus employed, has been properly objected to, but as the term visceral is currently applied, not only to all of these arches themselves, but also to their inner cartilaginous bars, there would seem to be no good reason for not applying it also to the external cartilaginous bars. But as, with equal reason, the branchial and branchiostegal rays would then have to be collectively called the visceral and viscerostegal rays, I retain the term extrabranchial for these cartilages in whatever arch they may be found. Branchial, hyal and visceral I employ as proposed by Gaupp ('05).

The vena jugularis, which seems to have had a markedly important influence on the development of the definitive cartilaginous bars of the prebranchial visceral arches, is not always, in the different orders of fishes, formed by the fusion of identical sections of the venae cardinalis anterior and capitis lateralis of embryos, the former vein being the primitive one and being said to lie ventro-mesial to the roots of all of the cranial nerves, while the latter one is of secondary formation and is said to lie dorso-lateral to those roots (Hochstetter '06). The definitive vein also has, in certain fishes, different relations to the hyomandibula, as I have quite recently had especially called to my attention, lying dorso-external to the hyomandibula in *Chlamydoselachus* but ventro-internal to that element in *Amia* and teleosts (Allis '14 b, p. 235). Wishing to know if these differences in the relations of the vein to the cranial nerves and to the hyomandibula were in any way related to each other, I have had the relations of the vein to the trigeminus and posttrigeminus nerves traced in a certain number of fishes.

In the *Selachii* and *Batoidei* the definitive vena jugularis is said by Hochstetter ('06) to be formed entirely by the vena capitis lateralis and to agree in this with the definitive vein in *Tropidonotus* and the *Mammalia*. In *Mustelus* (probably *laevis*) I find the vein lying dorsal to all of the components of the nerves here under consideration, excepting only the latero-sensory fibers which, in their exit from the cranium and in their peripheral distribution, are associated with the nervi trigeminus, glossopharyngeus and vagus. The vein lies ventral to all of these latero-

sensory nerves, but it lies dorsal to the latero-sensory fibers that issue with and as a part of the nervus hyomandibularis facialis.

In the Teleostomi, Hochstetter does not give either the method of development or the composition of the definitive vena jugularis. I find this vein, on one side of the head of a 43 mm. embryo of *Amia calva*, running posteriorly ventral to the ganglion on the main root of the nervus trigeminus and then upward between that ganglion and the ganglion on the root of the nervus facialis; then posteriorly dorsal to the latter ganglion, and dorsal also to the latero-sensory fibers which issue with the nervus hyomandibularis facialis, but ventral to the latero-sensory fibers which issue with the nervus trigeminus; then downward between the nervi facialis and glossopharyngeus; and then posteriorly ventral to the latter nerve and the vagus: the first section of the vena capitis lateralis that is formed in this fish thus corresponding to the one said by Hochstetter to be first formed in reptiles. On the other side of the head of this embryo of *Amia* the vena jugularis had a similar course, but a large branch of it passed dorsal to the nervus glossopharyngeus and then downward between that nerve and the vagus to fall again into the main vein, this thus showing a second section of the vena capitis lateralis in process of formation. In one adult specimen of *Amia* I find the vein running ventral to the nervus trigeminus, dorsal to the nervi facialis and glossopharyngeus but ventral or lateral, and hence morphologically ventral, to the latero-sensory nerves which issue with the nervi trigeminus and glossopharyngeus, and then ventral to the nervus vagus. The definitive vena jugularis of *Amia* is thus formed by the trigeminus and vagus sections of the vena cardinalis anterior and the facialis and glossopharyngeus sections of the vena capitis lateralis, and it corresponds, not only to the second stage in the formation of the definitive vein in reptiles, as given by Hochstetter, but also to the definitive vein (vena petroso-lateralis) of amphibians as given by Drüner ('04). The conditions in the one embryo examined would seem to show that no other sections of the vena capitis lateralis are ever formed in *Amia*.

In an 80 mm. specimen of *Lepidosteus osseus* and a 141 mm. specimen of *Polyodon spathula* the vena jugularis has the same relations to the several cranial nerves that it has in *Amia*, and I find similar conditions in young specimens of *Hiodon tergisus*, *Scorpaena scrofa* and *Cottus aspera*, and in the adults of *Scombrex saurus*, *Gadus merlangus* and *Trigla hirundo*. In a 57 mm. specimen of *Catostoma occidentalis* and a 40 mm. specimen of *Gastrosteus aculeatus* the glossopharyngeus section of the vein was the only one which lay dorsal to the related nerve. In an adult *Cyprinus carpio* and a 48 mm. specimen of *Carassius auratus* the facialis section of the nerve alone had this position. In embryos and the adult of *Ameiurus nebulosus* the vein lay ventral to all of the four nerves here under consideration, the entire vena cardinalis anterior thus here persisting as the definitive vein; *Ameiurus*, and hence probably all of the *Siluridae*, thus differing from other teleosts in this respect as well as in the arrangement of the pseudobranchial and carotid arteries, the innervation of the recti muscles of the eye-ball, and the condition of the myodome (Allis, '08, '09).

In *Ceratodus* embryos of Semon's Stage 45, Greil ('13, figs. 2-3, pl. 51) shows the vena jugularis, called by him the capitis lateralis, lying ventral to the nervus trigeminus and dorsal to the nervi facialis, glossopharyngeus and vagus, but, as in the *Plagiostomi* and *Teleostomi*, ventral to the latero-sensory nerves which issue with the nervi trigeminus, glossopharyngeus and vagus. In Stage 48 of this fish (l.c. fig. 2, pl. 55) the vein apparently still lies ventral to the nervus trigeminus and the same latero-sensory nerves, but it here lies ventral also to the nervus facialis, though still dorsal to the glossopharyngeus and vagus. This must, accordingly, be a less advanced stage than that shown in the embryo of Stage 45, unless it be that the vein change, a second time, its relation to the nervus facialis. But however this may be, it is evident that the definitive vein in this fish is of the *plagiostoman* and *reptilian* (*Lacerta*) type rather than the *teleostean* or *amphibian*.

One further feature of this vein may here be mentioned. The large orbital venous sinus, found so well developed in the *Plagi-*

ostomi, surrounds the nervus opticus, and the nervus ophthalmicus profundus traverses it in its course through the orbit, thus in a measure being also surrounded by it. This sinus would thus seem to correspond to that stage in the development of the posterior sections of the vena capitis lateralis which Hochstetter (l. c., p. 133) describes as a 'Veneninsel' surrounding the related segmental nerve. A crescentic sinus is found, in the Plagiostomi, similarly related to the nervus olfactorius.

These several variations in the relations of the vena jugularis to the cranial nerves, while they emphasize the facts that the Plagiostomi form a group wholly apart from the other gnathostome fishes and that the Siluridae are similarly grouped apart from the other Teleostei, do not present any features which indicate that they have in any way influenced the development of the hyomandibula, as will be later evident. They can accordingly be neglected in the present discussion. They however justify the use of the term vena jugularis rather than either of the terms vena cardinalis anterior or vena capitis lateralis, both of which are frequently used, and the terms vena petroso-lateralis or petrosa lateralis, introduced by Drüner ('01) for the corresponding vein in the Urodela and accepted by Kingsbury and Reed ('09) as eminently appropriate for those animals, might, at present, be confusing if applied to fishes.

In *Chlamydoselachus* the pharyngobranchials lie imbedded in what has, in one of three specimens examined, quite markedly the appearance of a continuous sheet of muscle fibers which is in process of differentiation into the Mm. interarcuales dorsales I of Vetter's ('74) descriptions of other selachians. This muscle-sheet lies immediately internal (dorsal) to the lining membrane of the branchial cavity, extends from the hyal to the most posterior branchial arch, and, in the one specimen above referred to, its mesial edge lay everywhere slightly mesial to the dorso-mesial ends of the pharyngobranchials. The muscle fibers all run postero-mesially, the muscle-sheet being considered, for convenience of description, to run from in front posteriorly, and as the pharyngobranchials do not extend entirely across the sheet they have the appearance of being intercalated obliquely in the

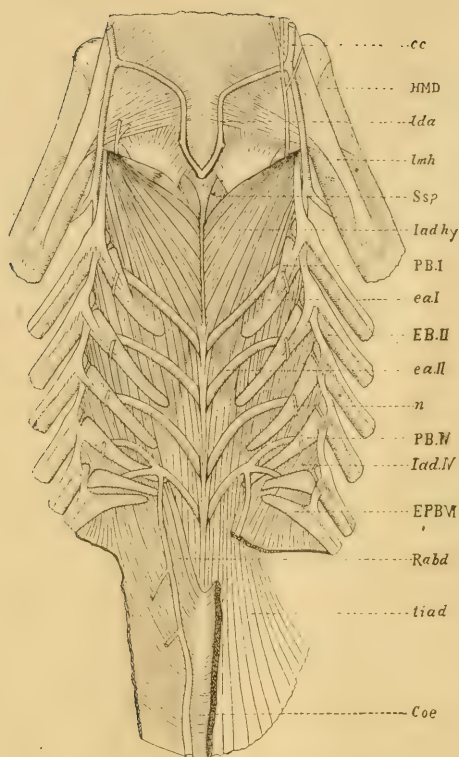


Fig. 1 Ventral view of the roof of the branchial chamber of *Chlamydoselachus anguineus* after the lining membrane of the chamber has been removed, showing the pharyngobranchials, efferent branchial arteries and interarcuales dorsales muscles in natural position.

ABBREVIATIONS

cc, common carotid artery
Coe, constrictor of the oesophagus
ea.I, efferent branchial artery of first branchial arch
ea.II, efferent branchial artery of second branchial arch
EB.II, epibranchial of second branchial arch
EPB.VI, epi-pharyngobranchial of sixth branchial arch
HMD, hyomandibula
Iad. IV, M.interarcualis dorsalis between arches IV-V
Iad.hy, M.interarcualis dorsalis between arches hy-I

lda, lateral dorsal aorta
lmh, ligamentum mandibulo-hyoideum
n, cut ends of nerves to tissues of roof of branchial chamber
PB.I, pharyngobranchial of first branchial arch
PB.IV, pharyngobranchial of fourth branchial arch
Rabd, retractor arcuum branchialium dorsalis
Ssp, subspinalis muscle
tiad, ligamentous sheet formed by tendons of Mm. interarcuales dorsales

course of the muscle fibers. Those fibers that have their origins from the dorso-mesial (proximal) portion of a pharyngobranchial, pass mesial to the dorso-mesial (proximal) end of the next posterior pharyngobranchial and are inserted on a series of long and slender tendons which run posteriorly, nearly parallel to the vertebral column. These tendons lie in and form part of a thin but strong sheet of ligamentous tissue which extends posteriorly considerably beyond the branchial chamber and is there attached to the tough fascia covering the ventral surface of the dorsal muscles of the trunk. The ligamentous sheet here lies between the trunk muscles and the constrictor of the oesophagus, but separated from the latter muscle by a median V-shaped muscle which has its origin from the ventral surface of the vertebral column and is inserted, on either side, on the closely adjoining dorso-mesial (proximal) ends of the fifth pharyngobranchial and the so-called sixth epibranchial (Garman, '85), and in part also on the fourth pharyngobranchial. It is continuous, along the greater part of its lateral edge, with the constrictor of the oesophagus, and is evidently in process of differentiation from that muscle. I have not yet determined its innervation, but its position and its relation to the constrictor are such that it seems quite unquestionable that it is innervated, as that muscle is, by branches of the nervus vagus. This muscle, and not one or more of the intercuales dorsales muscles, is then quite certainly the homologue of the retractor arcuum branchialium of *Amia* and teleosts, and Edgeworth's ('11) conclusion that this retractor muscle of *Amia* and teleosts is derived from trunk myotomes is probably in error. If this be so, one more of the instances frequently cited in support of the view that muscles are subject to radical changes in the manner of their innervation is apparently disposed of.

The mesial edge of the ligamentous sheet above described is strongly attached, throughout its entire length, mainly to the ventro-lateral corner of the vertebral column, immediately lateral to a shallow median groove which lodges the lateral dorsal aorta, but in part, also, to the ventral surface of a tough membrane, continuous with the ligamentous sheet, which extends

from this line of attachment across the median groove to the corresponding line on the opposite side of the head, thus enclosing and protecting the dorsal aorta.

That portion of the muscle-sheet which lies anterior to the first pharyngobranchial, forms about one-half the length of the sheet, and, there being no pharyngohyal, the muscle fibers here all have their origins from a stout ligamentous band extending from the dorsal (proximal) end of the hyomandibula to the dorsal (proximal) end of the first epibranchial, the band gradually diminishing in thickness and consistence toward its hind end. The posterior fibers of this part of the muscle-sheet have their insertions, as do the fibers of the interbranchial portions of the sheet, on the anterior edge of the next posterior pharyngobranchial, which is, in this case, the first pharyngobranchial. A small bundle of fibers immediately anterior to these posterior ones then passes mesial to the dorso-mesial end of the first pharyngobranchial, and its fibers, becoming tendinous, join and form part of the ligamentous sheet above described. The remaining fibers of this anterior portion of the muscle, which fibers form the larger part of it, are inserted in part directly on the ventro-lateral corner of the anterior portion of the vertebral column and in part on an anterior extension of the median subaortal membrane, the latter fibers reaching the median line of the head and there being in contact with their fellows of the opposite side.

In the other two specimens that were examined the conditions differed from those above described only in that the muscle-sheet was narrower, and that the pharyngobranchials, excepting the first, extended entirely across it. Those bundles of fibers that, in the one specimen, passed mesial to the dorso-mesial ends of the pharyngobranchials were accordingly wanting in the two other specimens, excepting only the bundle that had its origin from the first pharyngobranchial. The related ligamentous sheet nevertheless existed in these two specimens as in the other one, and extended the full length of the branchial region, but it had unfortunately been cut and partly dissected away before attention was called to it and its mesial and posterior attachments could not be determined. Those attachments were however

quite unquestionably the same as in the specimen first described. In all three specimens the anterior portion of the muscle-sheet was wider and stronger than the posterior portions, and it was here, only, that the muscle fibers reached the middle line of the body.

In the specimen first described a small and quite distinctly separate bundle of muscle fibers had their origins from the ventro-lateral edge of the hind end of the neurocranium, and, running directly posteriorly, were inserted on the anterior edge of the large muscle-sheet close to its mesial end. This little bundle of fibers was not found in the other two specimens, but in them certain of the anterior fibers of the large muscle-sheet were themselves inserted on the ventro-lateral edge of the hind end of the neurocranium.

This large muscle-sheet, considered as a whole, is thus attached, both anteriorly and posteriorly, to fixed, or relatively fixed (hyomandibula) points, and hence can not act, as a whole, either as a protractor or a retractor of the branchial arches. It can however act to draw the pharyngobranchials closer together and hence have either a protractor or retractor action on individual arches. It must also have a levator action on all the arches, for it is strongly attached to the neural axis. The hyobranchial portion of the sheet, the portion that lies between the hyal and first branchial arches, is certainly simply an anterior member of the *Mm. interarcuales dorsales I* of Vetter's descriptions of other selachians, and it would seem as if the little anterior bundle of fibers found in one of the three specimens might be a remnant of a prehyal, or hyomandibular, portion of the sheet. The hyobranchial portion of the sheet would seem to be the homologue of the subspinalis muscle of Vetter's and Marion's ('05) descriptions of *Acanthias*, which has its origin on the hind end of the neurocranium and its insertion on the dorso-mesial end of the first pharyngobranchial, while the small hyomandibular portion, found in one specimen, would seem to be the homologue of the subspinalis muscle of Vetter's descriptions of *Heptanchus*, which has its origin on the ventral surface of the hind end of

the neurocranium and its insertion on the ventral surface of the anterior portion of the vertebral column.

The antero-mesial edge of the muscle-sheet is practically parallel with the pharyngobranchials, and at once suggests that a pharyngohyal must primarily have existed there. There are, however, no special tissues that seem to represent rudiments of that element. Attached to the interarcual ligament which gives origin to this part of the muscle, near its anterior end, there was, in one specimen, a small and delicate piece of cartilage, which is apparently one of the interarcual cartilages, to be later described, and not a rudimentary pharyngohyal.

The efferent branchial arteries, in all three specimens, perforated the median subaortal membrane and ran antero-laterally across the ventral surface of the muscle-sheet to its lateral edge, each artery reaching the edge immediately anterior to the pharyngobranchial of the arch to which it belonged and slightly mesial to the distal end of that pharyngobranchial. The artery then ran outward across the anterior edge of the pharyngobranchial, crossed the dorsal (external) surface of that cartilage and so, having passed dorsal (external) to the related dorsal interarcual ligament, reached the external surface of the epibranchial of its arch. The vena jugularis lay everywhere dorsal to the sheet of muscular and ligamentous tissues and hence dorsal also to the pharyngobranchials.

The pharyngobranchials of *Chlamydoselachus* thus lie in a sheet of muscular and ligamentous tissues which, although it lies immediately internal (dorsal) to the lining membrane of the branchial chamber, is separated from that lining membrane by the efferent branchial arteries. The latter arteries therefore lie, in a part of their course, ventral (internal) to the pharyngobranchials, and as these latter cartilages are universally considered to lie primarily internal to the arteries it seems quite certain that, in *Chlamydoselachus*, the dorso-mesial ends of the pharyngobranchials became secondarily attached to the muscle-sheet, which primarily lay dorsal to them as well as to the efferent branchial arteries, and were in consequence lifted upward dorsal both to the latter arteries and to the dorsal aorta. If this

muscle-sheet were then to abort, the pharyngobranchials still retaining their attachment to the neural axis, the dorso-mesial ends of those cartilages would still lie dorsal both to the efferent branchial arteries and the dorsal aorta. But, if the attachment to the neural axis had not been acquired, or had been lost, those ends of the pharyngobranchials would lie ventral to the arteries, and morphologically ventral also to the dorsal aorta. In either case both the pharyngobranchials and the muscle-sheet, if it persisted, would lie definitely ventral to the vena jugularis, their relations to that vein not having been in any way disturbed. And this is what I find in the very unsatisfactory descriptions of other fishes that I have at my disposal.

In *Stegostoma*, Luther ('09) says that the epibranchials of the first and second branchial arches come into contact with the neurocranium ventro-mesial to the hyomandibular articulation, and hence, as will be later shown, certainly ventral to the vena jugularis. The relations to the lateral dorsal aorta are not given. In *Ceratodus*, Krawetz ('10) says that the epibranchial of the second branchial arch often comes into contact with the auditory capsule, and reference to Greil's ('13) figures will show that the point of contact must certainly lie dorso-lateral to the arteria carotis interna, which is the anterior prolongation of the lateral dorsal aorta, and ventral to the vena jugularis. In *Amia*, I (Allis, '97, fig. 61, pl. 36) found the dorsal (proximal) end of the pharyngobranchial of the first branchial arch attached to the neurocranium dorsal to the common carotid artery (lateral dorsal aorta) and ventral to the vena jugularis, the pharyngobranchials of the other branchial arches all lying ventral to the aorta as well as to the vena jugularis. In *Scomber* (Allis, '03) the dorsal end of the pharyngobranchial of the first branchial arch is also in contact with and attached to the neurocranium dorsal to the lateral dorsal aorta and ventral to the vena jugularis, and, as the dorsal end of the first pharyngobranchial of certain of the Clupeidae (Ridewood '04) comes into contact with the neurocranium immediately ventral to the trigemino-facialis chamber, through which the vena jugularis undoubtedly passes as it does in *Amia* and *Scomber*, the pharyngobranchial must there have

the same relations to the vein and artery that it has in Scomber. In *Anmocoetes* the dorsal ends of the branchial bars have fused with the neural axis, and Favaro ('08) shows the dorsal aorta lying ventral to them, and the vena jugularis and its anterior prolongations—the *venae jugularis dorsalis* and *capitis lateralis*—dorsal to them; and I find similar relations in a single specimen of *Petromyzon* that I have had examined. These so-called external cartilaginous arches of these fishes thus have the same relations to these two important blood vessels that the so-called inner cartilaginous arches of the gnathostome fishes have.

These relations of the so-called inner cartilaginous bars of the branchial arches to the aorta and vena jugularis are thus quite unquestionably not only a common feature but also a fundamental characteristic of all fishes, and, as it seems unquestionable that the prebranchial arches of the gnathostome fishes were primarily similar to the branchial ones, they must have been a primary characteristic of those arches also. These relations have, in fact, persisted in both the hyal and mandibular arches of all the Plagiostomi, so far as I can find described and as will be later shown, and because of this, and also because all apparent deviations from the rule in the prebranchial arches of other gnathostome fishes can be fully explained by the assumption of the association, or fusion, with the inner cartilaginous bars of those arches, of the related extrabranchial or interarcual cartilages, I assume, as stated in the opening paragraph of this paper, that they have persisted and are invariable in all gnathostome fishes in so far at least as the vena jugularis is concerned, which is the important consideration in this discussion. But, before considering these prebranchial arches, the extrabranchial and interarcual cartilages of the branchial region must be considered.

The extrabranchials are generally considered to be peculiar to the Plagiostomi and to be specially modified dorsal and ventral ones of the branchial rays of that arch in the diaphragm of which they lie (K. Fürbringer '03). They are said to differ radically from the other branchial rays in being attached, respectively, to the pharyngeal and hypal, instead of to the epal and ceratal elements of the inner arches, and this attachment is to those ele-

ments of the arch next posterior to the one in the diaphragm of which they lie instead of to the elements of their own arch. Dohrn ('84, p. 119) says that they never articulate with the elements to which they are attached, which doubtless means that their bases do not reach and come into contact with those elements, but Fürbringer (l. c., p. 428) calls attention to the fact that, while this is true of most of the Plagiostomi, it is not true of *Laemargus*. Fürbringer does not say with which element of the arch the extrabranchial of *Laemargus* comes into contact, but Gegenbaur's ('98, fig. 270) reproduction of White's figure of a branchial arch of this fish shows the contact with the dorsal end of the epibranchial. But however this may be, the important consideration in this discussion is that the conditions in *Laemargus* definitely show that the extrabranchials can come into contact with some element of the inner cartilaginous bar of the arch next posterior to the one to which they belong; and if they be modified branchial rays, as is generally considered to be the case, it is evident that they have potentially the possibility of coming into contact with some element of their own arch and of there fusing with it, as the branchial rays of *Torpedo* actually do. According as an extrabranchial is attached to an element of its own arch or to one of the next posterior arch, its relations to the nerve and artery of the arch to which it is so attached change from posterior and superior in the former case to anterior and inferior in the latter, this at once suggesting the suprapharyngobranchials and infrapharyngobranchials of van Wijhe's ('82) descriptions of ganoids and *Polypterus*; and it was the evident suggestion of the extrabranchials representing one or the other of these two elements, both of which were considered by van Wijhe to belong to and form part of the inner cartilaginous bar of a complete and normal branchial arch, that led me to undertake the present study. And it is perhaps needless to say that had I known how far it would lead me I should not have undertaken it with the limited material at my disposal.

The extrabranchials are said to be developed either in supporting and protecting relation to the branchial diaphragms, as are the other branchial rays (Dohrn '84), or as a special adapta-

tion of certain of those rays to the purpose of keeping the branchial clefts open and so preventing a possible stoppage of the flow of water through them (Fürbringer '03, p. 435). According to Gegenbaur ('72, pp. 164-166) they are archaic and very variable structures, inherited from the common ancestor of the Selachii and Cyclostomata and now in process of reduction and disappearance, and, as rudiments, only, of the dorsal ones were frequently found by him when the ventral ones were well developed, Gegenbaur concluded that the dorsal ones were subject to reduction before the ventral ones. Fürbringer ('03, p. 432), on the contrary, concludes that the dorsal extrabranchials, although almost always less strongly developed than the ventral ones, persist longer. Gegenbaur says that both dorsal and ventral extrabranchials are wanting in most of the Batoidei, for excepting rudiments, only, in *Rhynchobatus* and *Trygon*, he failed to find them in any of these fishes. Parker ('76), also, says that extrabranchials are wanting in the Batoidei, so far as he can make out, and he only describes ventral ones in *Scyllium canicula*. Ridewood ('97) has however since called attention to the fact that both dorsal and ventral extrabranchials were described by Rathke, in 1832, in *Raia aquila* and *Scyllium canicula*, and Foote ('97) has described them in *Raia erinacea*, *Raia radiata*, *Torpedo ocellata* and *Trygon pastinaca*.

In *Chlamydoselachus* I find the extrabranchials only slightly developed, as Fürbringer ('03) has already described them.

In *Mustelus* (probably *laevis*) I find the bases of the dorsal extrabranchials expanded into relatively large plates which lie directly against the dorso-lateral portion of the large venous sinus formed by the branchial portion of the vena jugularis, imbedded in the connective tissues surrounding that vein and evidently specialized, if not developed, in protective relation to the vein. They do not reach the inner branchial cartilages by a considerable interval, but they are connected with them by the connective tissues in which they are imbedded and by a narrow band-like muscle related to each extrabranchial. No special ligaments were found binding or connecting the two sets of cartilages. The enlarged bases of the ventral extrabranchials lie

ventro-mesial to the vena jugularis inferior, separated from that vein by certain muscles of the region but still in protective relation to it. The vein here lies between the base of the extrabranchial and the base of the adjacent ventral ray of the branchial series, definitely separating the one from the other; which must be a secondary adaptation if the extrabranchials are simply modified branchial rays.

In both *Heptanchus cinereus* and *Acanthias blainvillii* the bases of the dorsal extrabranchials lie, as they do in *Mustelus*, against the lateral wall of the vena jugularis, but in a single specimen of *Cestracion* that I have, the dorsal ends of the dorsal extrabranchials pass dorso-mesial to the vena jugularis and almost reach the pharyngobranchials mesial to that vein, thus lying against the dorso-mesial surface of the vein. The relations of the dorsal extrabranchials of *Cestracion* to the vena jugularis are thus similar to those of the ventral extrabranchials of *Mustelus* to the vena jugularis inferior. The enlarged bases of these dorsal extrabranchials of *Cestracion* all touch each other and are bound together by connective tissue, but they have not fused into a longitudinal bar such as Gegenbaur ('72) describes in his specimen of this fish.

In *Raia radiata* I find the cartilages described by Foote ('97) as dorsal extrabranchials much as she describes them, but they are less completely fused with the expanded outer ends of the branchial rays. Like Gaupp ('05, p. 897), I should have been inclined to consider this cartilage simply as a plate resulting from the fusion of the outer ends of the branchial rays, were it not that the dorsal (proximal) end of the cartilage presents two points, one of which is bound by ligament to the epibranchial of its own arch and the other to the pharyngobranchial of the next posterior arch. This dorsal end of the cartilage lies, as do the bases of the extrabranchials in the *Selachii* above described, against the lateral wall of the vena jugularis. The anterior cartilage of the series lies in the hyal arch.

No other *Plagiostomi* or other fishes were examined in this connection, but in the dissections of the few fishes above referred to, the dorsal interarcual cartilages to which reference has several

times been made were found developed in, or in relation to, the series of dorsal interarcual ligaments which extend from one branchial arch to the next, at or near the points where the pharyngobranchials and epibranchials articulate with each other.

In the one specimen of *Chlamydoselachus* examined there were none of these cartilages excepting only the one already referred to which lies in the ligament that extends from the hyal to the first branchial arch.

In one specimen of *Torpedo ocellata* there was an interarcual cartilage related to each of the interarcual ligaments, and there was also one in a ligament which extends from the first branchial arch to the ventral portion of the hind end of the neurocranium; this last ligament evidently being the one that primarily extended from the first branchial to the hyal arch.

In one specimen of *Raia radiata* there was, on one side of the head, an interarcual cartilage related to the ligament which extends from the first to the second branchial arch, this cartilage lying close against the hind edges of the pharyngobranchial and epibranchial of the first arch. On the other side of the head a process on the hind edge of the first pharyngobranchial corresponded exactly, in position, to the independent cartilage on the other side, and similar processes were found, on both sides of the head, on the pharyngobranchials of the more posterior arches. These processes thus apparently represent a series of interarcual cartilages that have each fused with the pharyngobranchial of the next anterior arch. On one side of the head of this specimen a small and independent bit of cartilage was found at the dorsal end of the epihyal, apparently representing the dorsal interarcual that lies between the hyal and first branchial arches. Similar cartilages are said by Gegenbaur ('72, p. 175) to be found in most of the Batoidei, and he considered each of them to represent a pharyngohyal that had been segmented off from the dorsal end of the secondary epihyal of the fish, the primary epihyal being included in the hyomanidubula and the secondary epihyal being formed by secondary segmentation from the dorsal end of the ceratohyal.

In one specimen of *Mustelus* (probably *laevis*) a small cartilage was found attached to the anterior edges of the articulating ends of the pharyngobranchial and epibranchial of the first branchial arch, this cartilage thus apparently being the interarcual cartilage that lies between the hyal and first branchial arches, and if so it is important in that it shows that these cartilages can be related either to the anterior or posterior one of the two arches between which they lie. No other independent interarcual cartilage was found on either side of the head, but there was a somewhat corresponding process on the anterior edge of the dorsal end of each epibranchial, similar to the processes shown by Parker ('76) in *Scyllium canicula*.

These interarcual cartilages and the related ligaments of selachians thus have exactly the relations to the branchial clefts and inner cartilaginous arches that the 'epitremal' longitudinal processes of the cartilaginous branchial arches of *Ammocoetes* and *Petromyzon* have (Gaupp '06), and if the cartilaginous arches themselves of these fishes are homologous, as seems so probable, it would seem as if the interarcual and epitremal cartilages must also be homologous, notwithstanding that the interarcual ligaments of selachians lie internal to the efferent branchial arteries while the epitremal processes of *Ammocoetes*, as shown by Favaro ('08), lie external to those arteries.

The conditions found in the fishes above described, notwithstanding the limited number that were examined, seem to warrant the conclusion that the extrabranchials of the Plagiostomi—whether dorsal or ventral, and whatever their origin—have had their basal portions either developed or specialized in protective relation to the related vena jugularis. And as these extrabranchial cartilages are presumably archaic structures, and as both dorsal and ventral ones are said to be found, in all the Plagiostomi, related to the hyal as well as to the branchial arches, there seems no reason to doubt, not only that they were developed also in the prehyal arch or arches of those fishes but also in the corresponding arches of the common ancestor of all the gnathostome fishes, and that, accordingly, rudiments or modifications of them should be found in the Teleostomi and Dipneusti. In

these latter fishes I have made no special search for these cartilages, but it seems to me practically unquestionable that, as already stated, they are represented in the suprapharyngobranchials of van Wijhe's ('82) descriptions of ganoids and *Polypterus*. These suprapharyngobranchials are shown by van Wijhe, sometimes as independent cartilages, sometimes fused with the infrapharyngobranchials, and sometimes fused with the epibranchials of their respective arches. In *Polyodon* van Wijhe did not find any of them, but Bridge ('79, p. 709) found, in each of the first two branchial arches of this fish, a little cartilage, described by him as a short pointed cartilaginous ray, directed upward and backward from the upper posterior angle of the related epibranchial; these so-called rays thus certainly being strictly similar to the cartilages described by van Wijhe as suprapharyngobranchials in *Acipenser*. The relations of the suprapharyngobranchials to the vena jugularis are not given either by van Wijhe or Bridge, but it would seem as if they must lie lateral to that vein. If so they are certainly extrabranchials, and it is evident that all the various forms of suprapharyngobranchials described by van Wijhe would arise by simple adaptations and fusions of such cartilages with one or the other of the inner cartilages of its arch, or with that cartilage together with the related interarcual. In *Amia* there are, furthermore, indications that certain of the ventral extrabranchials have been preserved, for in this fish there are ventro-mesial processes on the third and fourth hypobranchials, shown by both van Wijhe (l. c.) and myself ('97; fig. 50, pl. 33), which lie ventro-mesial to the vena jugularis inferior and hence in the same protective relation to that vein that the bases of the ventral extrabranchials have in the *Plagiostomi*. A similar process is also shown by van Wijhe on the third hypobranchial of *Acipenser*. The so-called suprapharyngobranchial of the first branchial arch of my descriptions ('03) of *Scomber* I now consider to be an interarcual and not a suprapharyngeal (extrabranchial) cartilage.

The pharyngeal and hypal elements of the branchial arches of the *Plagiostomi* all project postero-mesially, instead of antero-mesially in the lines prolonged, respectively, of the epal and

ceratal elements of those arches. This sigma-form of arch is said by Gegenbaur ('72) to be peculiar to these fishes, but in the *Holocephali* (Schauinsland '03), and, as will be later shown, possibly also in *Ceratodus*, the pharyngobranchials project postero-mesially as they do in the *Plagiostomi*. In teleosts also, the pharyngobranchial of the first branchial arch, when it comes into articular relations with the neurocranium, may be directed dorso-postero-laterally at a sharp angle to the epibranchial (*Scomber*, *Clupeidae*), strongly recalling the conditions found in the *Plagiostomi*, but differing radically from the conditions in those fishes in that the pharyngobranchial is directed dorso-postero-laterally along the lateral surface of the neurocranium instead of postero-mesially beneath the vertebral column.

Gegenbaur considered this sigma-form of arch to be a secondarily acquired but archaic feature, for he found it even in young embryos of *Acanthias*; and he thought it caused by the dorsal ends of the branchial arches having been pushed posteriorly by repeated acts of deglutition. But if this be the cause there seems no good reason why it should not have been equally operative in all fishes, and, furthermore, it certainly could not have caused the conditions just above described in *Scomber* and the *Clupeidae*. In these latter fishes it seems certain that the epal and ceratal elements of the first branchial arch have been carried forward beyond a point where the pharyngeal element of that arch had previously become attached to the neurocranium, instead of the dorsal ends of the pharyngobranchials having been pushed backward; and as the dorsal ends of all of the pharyngobranchials are, in *Chlamydoselachus*, and hence probably in other selachians also, attached to the vertebral column and prevented from shifting forward by the intervening roots of the efferent branchial arteries, it seems to me equally certain that here also the epal and ceratal elements have been carried forward, doubtless because of the relatively marked anterior growth of the chondrocranium, instead of the dorsal ends of the pharyngobranchials having been pushed backward. Where the dorsal ends of the branchial arches were not so attached to the neurocranium or vertebral column, as in all or most of the arches

in the Teleostomi, the arches were carried bodily forward and the sigma-form of arch was not acquired. But whatever the cause may have been of the sigma-form of the branchial arches in the Plagiostomi, it is certain, as will be later shown, that this form was also impressed upon the dorsal end, at least, of the cartilaginous bar of the hyal arch of these fishes, and probably also upon that end of the bar in the mandibular arch.

Parker ('76, p. 211) says that the pharyngobranchials of *Scyllium canicula* normally turn backward, "the opposite direction to that taken by the hyoid and mandibular arches," but having great mobility, they "may turn forward:" and he adds that, in the branchial arches, he has "figured them both ways for illustration." Unless *Scyllium* differs markedly from *Chlamydoselachus* in that the dorsal ends of the pharyngobranchials are suspended loosely beneath the vertebral column, or not at all attached to it, this great mobility of these cartilages must be limited to the articulation with the epibranchial, and the pharyngobranchials could only be directed forward as the result of a shifting posteriorly of the entire branchial apparatus. And unless *Scyllium* also differs markedly from both *Stegostoma* and *Chlamydoselachus*, as will be shown immediately below, the dorsal end of the hyal arch is certainly directed backward and not forward. Furthermore, Haddon ('87, p. 208), in a figure said to be copied from Marshall, shows the pharyngobranchials of *Scyllium canicula* directed backward at such a marked angle to the epibranchials that it seems almost impossible that they could ever be directed forward, and Gegenbaur ('72) shows them directed backward in *Scyllium catulus*. The conditions shown in Parker's figure giving a lateral view of the skull and branchial arches of the adult *Scyllium canicula* must then be exceptional, and it is to be regretted that this particular figure has been reproduced and perpetuated in so many text books (Parker and Betany, Cambridge Natural History, Wiedersheim, etc.).

The hyomandibula can now be considered, and it will be best to begin with the Selachii.

Gegenbaur ('72, p. 175) considered the so-called hyomandibulae of the Selachii, Batoidei and Teleostei (and doubtless also

of all the other Teleostomi) to be strictly homologous cartilages, and he apparently considered the single cartilage to represent the entire dorsal half of the cartilaginous bar of the hyal arch and hence to be the serial homologue of the combined epal and pharyngeal elements of the branchial arches.

Parker ('76, p. 199) says that the hyomandibula of the Selachii represents "the whole of the upper part of the hyoid arch;" that "Here, then, the 'pharyngohyal' and the 'epihyal' are in one piece, and the 'ceratohyal' and 'hypohyal' are in one;" and (p. 205) that the suspensorial part of the hyal arch "is morphologically a whole 'epihyal' piece, with no pharyngohyal segment above, and articulating with a 'ceratohyal' from which no 'hypohyal' element has been cloven." This would certainly seem to mean that Parker considered the hyomandibula to contain the unsegmented pharyngeal and epal elements of the arch, and I have hitherto always so considered it, but as he also says (p. 211) that the hyomandibula is the 'counterpart' of the epibranchials, and, in a later work ('82, p. 147), that "In the Dog-fish (*Scyllium*) the hyomandibula is evidently the serial homologue of the epibranchials," it is not clear just what his opinion was. The same uncertainty exists as to the branchial arches also, for after first saying ('76, p. 199) that these arches "break up into four pieces on each side, a 'pharyngo-', 'epi-', 'cerato-', and 'hypo-branchial' element," he later says (p. 211) that the pharyngobranchials chondrify separately and independently of the main bar. One thing, however, he very definitely states, without later contradiction or qualification; that there is no separate pharyngeal element in the hyal arch ('76, p. 211).

Dohrn ('84) implies, if he does not definitely say, that the cartilaginous bars of the branchial arches, in both the Selachii and the Batoidei, chondrify as a single piece; that these bars later segment transversely, at the middle of their lengths, forming so-called epibranchial and ceratobranchial elements; and that still later the pharyngobranchials and hypobranchials are segmented off, respectively, from the dorsal ends of the epibranchials and the ventral ends of the ceratobranchials. In the dorsal half of the hyal arch this second segmentation is said not to take place,

and this is confirmed by his statements, in a later work ('85, p. 13-14), that no true pharyngeal element is developed in this arch, and that the presence of an extrabranhial in the arch shows that the pharyngeal element is included in the hyomandibula. From these statements it is evident that the hyomandibula, both of the Selachii and Batoidei, must have been considered by Dohrn to be an epi-pharyngohyal, and as there are, according to him ('85), two visceral arches represented in the so-called hyal arch of these fishes, the epi-pharyngohyals of two adjoining arches must have fused to form the definitive hyomandibula of the adult selachian. This fusion of two arches here has not been generally accepted, and there is much question as to whether the cartilaginous bars of the visceral arches of living fishes chondrify as a single piece or as four separate elements (see Gaupp '05); but that the hyomandibula of the Selachii contains the unsegmented epal and pharyngeal elements of the arch has, I believe, always been the generally accepted opinion.

Schauinsland, however, in 1903, came to the conclusion that the three pieces which form the cartilaginous bar of the hyal arch of *Callorhynchus* are ceratal, epal and pharyngeal elements, and that the epal element (das Epibranchiale) is certainly the homologue of the hyomandibula of 'other selachians,' the expression other selachians evidently meaning both the Selachii and the Batoidei. This is then a marked departure from Dohrn's conclusion, just above given, for the selachian hyomandibula would then be a simple epal element. Gaupp was not at that time ('05, p. 839) inclined to accept this conclusion, and said that, in any event, the question needed further special investigation. But Luther ('09, p. 13) later found a pharyngeal element as a distinct and separate cartilage in *Stegostoma tigrinum*, *Mustelus* (probably *laevis*) and *Galeus galeus*. In *Stegostoma* the element is said to be formed by two small cartilages which lie postero-mesial to the upper end of the hyomandibula, in a line practically parallel with the pharyngeal elements of the branchial arches. They are said by Luther to be attached to each other by a strand of connective tissue, to be firmly bound to the hyomandibula, and to help in the attachment of that cartilage to

the neurocranium. No association of these cartilages with the muscles or ligaments of the region is mentioned by Luther, but Gadow ('88) describes ligaments similar to those found in *Chlamydoselachus*, but no cartilages, in *Heptanchus*, *Oxyrhina* and *Sphyrna*. In each of these fishes two ligaments are said to have their origin from the dorsal end of the hyomandibula and, running posteriorly, to have their attachments, one to the dorsal end of the ceratobranchial of the first branchial arch and the other to the epibranchial of the same arch at about the middle of its length; but it must be that it is the epibranchial and pharyngobranchial of the arch, and not the ceratobranchial and epibranchial, that are here respectively meant.

From Schauinsland's, Luther's and my own observations, it thus seems certain that the hyomandibula of the Selachii is a simple epal element of the hyal arch, and the reason why this element, instead of the pharyngohyal, articulates with the neurocranium is found: in the attachment of the dorsal ends of the cartilaginous bars of the hyal and branchial arches beneath the vertebral column and hence posterior to the neurocranium; in the sigma-form which has been impressed, for some unknown reason, on the cartilaginous bars of these arches of these fishes; and in the fact that the pharyngeal elements of these bars apparently always articulate, not with the dorsal (proximal) ends of the epal elements, but with the posterior edge of the dorsal ends of those elements, leaving the dorsal ends themselves exposed (see Gegenbaur's figures). Because of these several conditions, when the auditory capsule began to develop, it came into contact with the sigma-shaped cartilaginous bar of the hyal arch at the dorsal bend in that bar, and when articulation with the neurocranium was formed it was necessarily with the exposed dorsal end of the epihyal. The pharyngeal element, impeded in its development by the bulging auditory capsule, then underwent reduction. The nerves and blood vessels of the region, with a single exception, retained unchanged their topographical relations to the epihyal (hyomandibula), if the conditions that I find in *Chlamydoselachus*, *Heptanchus* and *Mustelus* are typical of all the Selachii; for in these three fishes the lateral dorsal

aorta still lies mesial and hence ventral to the epihyal (hyomandibula), the vena jugularis lies dorsal to that element, the commissural vessel that forms the definitive afferent pseudobranchial artery (Allis '11 b, '12) crosses its external surface, and the nervus hyomandibularis facialis runs outward across its anterior edge to reach its external (dorso-lateral) surface, thus lying morphologically dorso-external both to it and to the pharyngohyal. The single exception to the rule is the efferent hyal artery. The efferent arteries of the branchial arches of these several fishes all cross the external surface of the distal end of the related pharyngobranchial in order to reach the external surface of the epibranchial. In the hyal arch the artery doubtless had primarily a similar course, but when that arch acquired articulation with the neurocranium by its exposed proximal end (which projected anteriorly beyond the efferent artery of the arch) the artery lay posterior to the articulating surfaces and hence passed over the posterior edge of the epihyal to reach its external surface.

The hyomandibula of the Selachii is, so far as I can determine from the literature and material at my disposal, always attached to the neurocranium by one or two more or less important ligaments in addition to the special articular ligaments. Ridewood ('95) describes both of these ligaments in *Scyllium* and calls them the superior and inferior postspiracular ligaments. The superior ligament is said by him to have its attachment on the auditory capsule dorsal to the vena jugularis ("vein connecting the orbital and anterior cardinal blood sinuses") and to be attached, ventrally, partly on the palatoquadrate and partly on the outer surface of the lower end of the hyomandibula. The nervus hyomandibularis facialis runs outward between the ligament and the hyomandibula, posterior to the one and anterior to the other. The inferior ligament is said to be attached by one end to a lateral projection at the base of the skull, below and behind the foramen for the nervus trigeminus, and by the other end to the postero-internal edge of the lower half of the hyomandibula, and partly also to the upper end of the ceratohyal.

Gegenbaur ('72) describes and figures the inferior postspiracular ligament in *Heptanchus* (1', fig. 1, pl. 15), without so naming

it, and he apparently shows it also in a figure (pl. 11, fig. 3) said to be of *Mustelus* (but probably *Galeus*?). The superior ligament is also described and figured by him, without being so named, in both *Mustelus* and *Galeus*, but in these two fishes only. Whether or not either of these two ligaments were found by him in other selachians can not be definitely told from the descriptions. Gegenbaur does not mention the relations of the superior ligament to the vena jugularis, but he makes the evident mistake of saying (l. c., p. 168) that the nervus hyomandibularis facialis passes through the slit-like space between the ligament and the hyomandibula and then continues onward, close against the cranium (dicht am Schädel), to the posterior surface of the hyomandibula. Gadow ('88) describes and figures one or both of the ligaments in *Heptanchus*, *Oxyrhina* and *Sphyrna*, but the figures and descriptions are of little value excepting in that they indicate that the ligaments are both generally found in the Selachii.

In embryos of *Mustelus laevis* I found, in 1901, both of these postspiracular ligaments, the superior ligament being in part attached, ventrally, to the dorsal edges of two diverticula of the spiracular canal, and I now find both of these ligaments in an adult specimen of this fish, the attachment to the spiracular canal not being, relatively, so markedly developed as in embryos. In *Chlamydoselachus* I find the inferior ligament strongly developed, while the superior ligament is represented by connective tissue strands which have their origin, partly on the postero-ventral surface of the postorbital process of the neurocranium, dorsal to the vena jugularis, and partly on the projecting anterior corner of the dorsal end of the hyomandibula and hence morphologically ventral to the vena jugularis. Running postero-ventrally, these two separate strands of tissue are apparently both attached wholly to the dorsal edge of the spiracular canal, but as the posterior wall of this canal is in part closely applied against and attached to the hyomandibula by intervening connective tissue, the two strands are doubtless continuous with this tissue and hence attached also to the hyomandibula. The superior postspiracular ligament of my embryos of *Mustelus* is thus here apparently in process of differentiation. In *Heptanchus* I find

the inferior ligament as described by Gegenbaur, and I also find a superior ligament extending from the cranial wall to the dorsal edge of the spiracular canal and hence similar to one of the two strands found in *Chlamydoselachus*. In *Lamna* I find both ligaments well developed, the inferior one being large and complicated and prolonged around the ventral end of the hyomandibula onto the mandibular cartilages. In *Cestracion* both ligaments are also found, and, in addition, the dorsal edge of the spiracular canal is connected with the cranial wall by a feeble strand of connective tissue.

These two ligaments, more or less developed, are thus probably found in nearly all, if not all, selachians, and they are such important structures that it would seem as if they must have their serial homologues in the branchial arches. Related to the dorsal ends of the branchial arches are interarcual ligaments and arcual and interarcual muscles. These arcual and interarcual muscles are of two kinds, the interarcuales dorsales I of Vetter's descriptions, called by M. Fürbringer ('97) the interbasales and said by Edgeworth ('11) to be derived from spinal myotomes, and the interarcuales dorsales II and III of Vetter's descriptions, called by Fürbringer the arcuales dorsales and said by Edgeworth to be derived from the branchial myotomes. The interarcuales dorsales II are short muscles which extend across the angle between the epibranchial and pharyngobranchial of an arch, and, if this muscle primarily existed in the hyal arch, it could readily have given origin to the inferior postspiracular ligament, the muscle losing its attachment to the aborting pharyngohyal and secondarily acquiring attachment to the neurocranium, and then becoming ligamentous. The superior ligament might have been derived either from the interarcualis dorsalis I which extended from the hyal to the mandibular arch; from the fascia of connective tissue that is associated with that muscle in *Chlamydoselachus* and has been already described; or from the corresponding dorsal interarcual ligament. In either case, because of the markedly dorsal position of the spiracular canal, this interarcual ligament would have been pushed upward toward the lateral wall of the neurocranium, and, passing lateral to the vena jugularis, could

have secondarily acquired attachment to the neurocranium dorsal to the vein. The ligament would in either case have lain primarily ventral to the nervus hyomandibularis facialis and hence when it had secondarily acquired attachment to the neurocranium it would lie anterior to that nerve.

In the Batoidei the conditions are markedly different from those in the Selachii.

Gegenbaur ('72) considered the hyomandibula of the Batoidei to be the strict homologue of that of the Selachii, the epihyal of his descriptions of the adult being formed by secondary segmentation from the dorsal end of the ceratohyal, and the pharyngohyal, where found, by segmentation from the dorsal end of the so-formed epihyal.

Parker, in his work published in 1876, says (p. 220) that the hyomandibula of *Raia* is formed by one half only of the epihyal, the primarily continuous cartilaginous bar of the hyal arch having been cleft obliquely, from the middle line upward and backward, instead of transversely at the middle line as in the Selachii. The anterior segment so cut off formed the hyomandibula and the postero-inferior one a bar which later segmented transversely into the definitive epihyal and ceratohyal. Parker (p. 214) calls this remaining, postero-inferior portion of the entire bar, before its secondary segmentation, the styloceratohyal, but as it is said to segment into an epihyal and a ceratohyal, it is not clear whether or not he intended to homologize the definitive so-called epihyal of the Batoidei with the stylohyal of the Teleostei. Parker repeatedly says, in this work, that no pharyngeal element is developed in the hyal arch of the Batoidei. But, in a footnote in a later work ('82, p. 147), he says of these fishes that "the metapterygoid and hyomandibular naturally classify themselves with the succeeding pharyngo-branchials;" which is not at all in accord with his earlier statements, and, as he makes no reference to those earlier statements, it is again not clear just what his opinion was.

Dohrn ('85) considered the hyomandibula of the Batoidei to belong to a visceral arch between the hyal and mandibular arches, and it is probable (l. c., p. 82) that he considered it to

represent the dorsal, epi-pharyngeal portion only of the cartilaginous bar of that arch. Van Wijhe ('01) says that it represents the dorsal half of the hyal, or first postmandibular arch, that the ventral half of that arch entirely aborts, and that the epihyal and ceratohyal of Gegenbaur's and Parker's descriptions of the adult represent the cartilaginous bar of a hyobranchial arch which lies between the hyal and first branchial arches.

In the adult of *Raia clavata*, Parker ('76) shows the hyomandibula in a position practically parallel with, but considerably ventro-lateral to, the pharyngobranchials. It articulates dorsally with the neurocranium, and in *Raia radiata* I find this articulation ventral to the vena jugularis and dorsal (lateral) to the lateral dorsal aorta. The ventro-anterior end of the hyomandibula is attached by ligament to the articulating ends of the mandibula and palatoquadrate, and this attachment is said by Parker (l. c., p. 220) to be primarily with the quadrate. The dorso-posterior end of the hyomandibula is shown much larger than the ventro-anterior one, and in addition to articulating with the neurocranium it is attached by a single ligament, both to the dorsal end of the epihyal and to the adjacent dorsal end of the epibranchial of the first branchial arch. Parker calls this the interhyal ligament, thus homologizing it with the ligament which, in ganoids and teleosts, connects the dorsal end of the ceratohyal, through the intervention of a cartilaginous interhyal (stylohyal), either with the symplectic or with the hyomandibula of those fishes, but he says that no cartilage is developed in the ligament in the Batoidei. There are no branchial rays related to the hyomandibula either in *Raia* or any others of the Batoidei, but there is a dorsal extrabranchial related to this arch.

In the adult of *Torpedo marmorata* (Gegenbaur '72) the conditions that it is important to here consider differ from those in *Raia* only in that there is a hook-like process on the anterior edge of the hyomandibula, and, as this process is also shown by Dohrn ('85, pl. 1, fig. 2 a) in his figures of embryos of *Pristiurus* in which the tissues are still in a procartilaginous condition, it is evidently an early acquisition in these fishes. In *Narcine* it is

said by Gegenbaur (l. c., p. 200) to be represented by a wholly independent piece of cartilage.

No ligaments are shown, in any of the Batoidei, connecting the cartilages of the mandibular arch with the epihyal or ceratohyal, by either Gegenbaur, Parker or Gadow ('88), and no postspiracular ligament, either inferior or superior, is shown by either of those authors. Both Parker and Gadow show a stout ligament connecting the ventro-anterior end of the hyomandibula with one or both of the mandibular cartilages, and Parker shows a stout ligament connecting the same end of the hyomandibula with the spiracular cartilage, called by him the metapterygoid. I also do not find an inferior postspiracular ligament in either *Raia* or *Torpedo*, but I find, in *Raia radiata*, the posterior wall of the spiracular canal lined with a tough ligamentous tissue which is firmly and directly attached, ventro-laterally (distally), to the anterior portion of the external surface of the hyomandibula, while dorso-mesially (proximally) a smaller and less consistent extension of the tissue passes dorso-external to the vena jugularis and is attached to the lateral wall of the auditory capsule. In *Torpedo ocellata* I find the hook-like process of the hyomandibula similarly connected by ligament with the auditory capsule, the process and ligament together evidently being the homologue of the ligament alone of *Raia*. This ligament of the Batoidei is thus quite certainly the homologue of the superior postspiracular ligament of the Selachii, and, like that ligament, the probable serial homologue either of the Mm. interarcuales dorsales I or the dorsal interarcual ligaments of the branchial arches of those fishes. The hook-like process of the hyomandibula of *Torpedo* and the corresponding independent cartilage of *Narcine* are then either simply chondrifications of that ligament, as I have already suggested in an earlier work ('01), or, and this seems much more probable, dorsal interarcual cartilages similar to those found in the branchial region.

If the conditions as thus described in the Batoidei be compared with those in the Selachii, it seems quite unquestionable that the hyomandibula of the former can not be the homologue of that of the latter, and it seems equally unquestionable that

the hyomandibula of the Batoidei is the pharyngeal element of its arch. The mouth and its bounding cartilages are, in the Batoidei, relatively transverse in position, and if this is not a primitive condition, it has been brought about by a shifting forward of the point of articulation, on either side of the head, of the cartilages of the upper and lower jaws, instead of by a shifting backward of the dorsal and ventral ends of those cartilages. The epal and ceratal elements of the branchial arches were not disturbed by this shifting, and retained their oblique position relative to the axis of the body, and, as the hyobranchial visceral cleft did not undergo an expansion proportional to the increased distance between the articular joints at the middle of the lengths of the first branchial and mandibular cartilaginous arches, the epihyal and ceratohyal, held in supporting relations to the anterior wall of the hyobranchial cleft, were pulled away from the mandibular cartilages. The pharyngohyal was not so held in place, for the pharyngeal elements of the branchial arches are never found in supporting relations either to the branchiae or the diaphragms of the related clefts. Furthermore, the dorsal ends of the hyal and branchial arches were apparently not pushed as far posteriorly in the Batoidei as in the Selachii, possibly because of the less pronounced cranial flexure in embryos of these fishes, and the thrust of the developing auditory capsule on the dorsal end of the pharyngohyal was lateral instead of posterior. This element of the hyal arch was accordingly in position to be utilized for the purpose of giving the hyostylic support to the mandibular cartilages that, in the Selachii, is given by the epihyal, and, as it was gradually pushed downward across the dorsal end of the epihyal, the latter element seemed to shift upward along its posterior edge in exactly the manner that Gegenbaur ('72, p. 175) assumes for these very cartilages, considered by him to be the epi-pharyngohyal and ceratohyal respectively. The pharyngohyal still retained its primary ligamentous attachments both with the epihyal and the epibranchial of the first branchial arch, through the interhyal and dorsal interarcual ligaments, and the conditions actually found in the adult *Raia* arose. The fact that it is a pharyngeal element and not an epal

element of the arch that thus came into supporting relations to the posterior wall of the spiracular cleft may explain the absence of branchiae on that wall of the cleft in these fishes, but this would not apply to the Selachii. In these latter fishes the absence of these branchiae may be correlated to the interposition of the superior postspiracular ligament between the cleft and the epihyal.

If this be the manner in which the cartilages of this arch have been developed, and there seems every reason to believe that it is, there can be no possible question of these cartilages representing two visceral arches, for the double concentration of mesoderm cells said by Dohrn ('85) to take place in this arch in embryos of these fishes would be fully accounted for; one center normally representing and giving rise to the pharyngohyal and the other to the remainder of the arch. There is also, under my assumption, no need to assume either that an important portion of the cartilaginous bar of a visceral arch has completely aborted in this region (van Wijhe, '01), or that a ventral segment (ceratohyal) of the arch has, by secondary segmentation, furnished a complete duplicate set of dorsal segments in its arch (Gegenbaur); and the break in the arch caused by the assumed change in position of the pharyngohyal would probably account for the difference in the muscles of the region as compared with selachians, a subject I have not yet been able properly to consider.

In the Holocephali, Hubrecht ('77) shows two cartilages in the dorsal half of the hyal arch, the smaller, dorsal one projecting postero-mesially, as the pharyngeal elements of the hyal and branchial arches do in the Selachii. Schauinsland ('03) shows a similar arrangement in an embryo of *Callorhynchus*, and, as already stated, he identifies the two cartilages as the epihyal and pharyngohyal, and says that the epihyal is certainly the homologue of the hyomandibula of other selachians (sicherlich homolog dem Hyomandibulare der Uübrigen Selachier). This, if I am right in my conclusions, is correct in so far as the Selachii are concerned, but incorrect for the Batoidei. In a specimen of *Chimaera colliei* which I have examined, neither the epihyal nor

the pharyngohyal, either articulate with or are attached by ligament to the neurocranium. In this specimen both these elements lie against the internal surface of the suspensorium, but separated from it by a delicate and highly pigmented membrane. The lateral dorsal aorta lies mesial to their dorso-mesial ends, the vena jugularis dorsal to them, and the nervus hyomandibularis facialis dorsal and anterior to them, as in selachians.

In all living elasmobranchs it is thus seen that the dorsal ends of both the pharyngeal and epal elements of the hyal arch always lie definitely dorsal (lateral) to the lateral dorsal aorta, ventral (internal) to the vena jugularis, and internal (ventro-postero-mesial) to the nervus hyomandibularis facialis, and I assume, as in part stated in the opening paragraph of this paper, that this relation of the inner cartilaginous bar of this arch to the artery, vein and nerve is invariable in all of the gnathostome fishes. In the Dipneusti (*Ceratodus*) and Teleostomi there is, however, a marked departure from the elasmobranchian type in that the definitive cartilaginous bar of this arch always either fuses or articulates with the neurocranium dorsal to the vena jugularis. It will be best to consider first the conditions in *Ceratodus*, because the development of this fish has recently been very carefully and fully described by several authors.

In the adult *Ceratodus*, Huxley ('76) described a cartilage which he considered to be "the homologue of the hyomandibular element of the hyoidean arch of other fishes;" this expression, in no way qualified by him, quite conclusively showing that he considered the hyomandibulae of these other fishes to all be homologous structures. He thought this cartilage in *Ceratodus* was probably the suspensorial tubercle of Günther's ('71) earlier descriptions, and it is said by him to lie internal to the operculum and to be firmly attached, by its anterior edge, to the skull at the point where the cranium proper passes into the suspensorium. The nervus hyomandibularis facialis is said to issue across its anterior edge. Van Wijhe ('82) confirms this relation of the nerve to the so-called hyomandibula, but adds that it seems to him not impossible that this latter cartilage may be an interhyal. Ride-wood ('94) finds both hyomandibula and suspensorial tubercle as

independent structures in his specimens, and says that the lower end of the hyomandibula, called by Huxley the symplectic process, is articulated to the dorsal surface of the tubercule, and that, as this tubercule gives attachment also to the hyosuspensorial ligament, the hyomandibula is thus indirectly connected with the ceratohyal. The nervus hyomandibularis facialis is said by him either to perforate the hind edge of the suspensorium or to issue between that hind edge and the anterior edge of the lower, process-like end of the hyomandibula, and this process-like end is said to be frequently found segmented off as a separate cartilage. In one of the several specimens examined by him, Ridewood (l. c., fig. 3 b, p. 637) found a little cartilage imbedded in the hyosuspensorial ligament which he says may "possibly have the value of an interhyal;" this little cartilage having been described by Pollard ('94) one month before and also said by him to probably represent an interhyal (stylohyal). Pollard (l. c.) concluded that Huxley's hyomandibula was an opercular cartilage, and Fürbringer ('04) is undecided as to whether it be such a cartilage (Kiemenstrahlenrudimente) or the homologue of the hyomandibula of other fishes.

Ridewood, alone, of the several authors above referred to, calls attention to the fact that the point where the so-called hyomandibula is attached to the neurocranium is widely separated from the auditory capsule, "in the vicinity of which the hyomandibular of fishes usually articulates." This intervening part of the neurocranium is, in fact, a wide concave surface presented ventro-latero-posteriorly, with the foramen faciale lying near its lateral margin and the foramen for the vena jugularis in its antero-ventro-mesial portion. The vena jugularis, after issuing from its foramen, runs posteriorly along the lateral surface of the neurocranium in the large jugular groove of van Wijhe's descriptions, which groove lies dorsal to the foramina for the glosso-pharyngeus and vagus nerves.

In a badly preserved specimen of *Ceratodus* that I have, I find, on one side of the head, this so-called hyomandibula with its symplectic process, and the ventral end of the process articulates with a stout, and slightly biconcave cartilage which, in

turn, articulates with the dorsal end of the ceratohyal. A mass of tough connective tissue that apparently includes the well defined hyosuspensorial ligament of Ridewood's descriptions binds these three cartilages together and also strongly attaches the ceratohyal to the hind edge of the suspensorium ventral to the symplectic process. The biconcave cartilage thus has markedly the appearance of a teleostean interhyal. The anterior edge of the symplectic process is bound to a slightly developed tubercular process of the suspensorium, and on its posterior edge there is a small independent cartilage, Ridewood's interhyal, which is attached to the postero-internal surface of the hyosuspensorial ligament and is also in contact with the biconcave cartilage. On the other side of the head of this specimen the hyomandibula is without symplectic process, and there is here no biconcave cartilage interposed between the hyomandibula and ceratohyal. The tubercular process of the suspensorium is larger than on the other side, and the hyosuspensorial ligament is attached mainly to it, but partly also to the ventral end of the hyomandibula. A small cartilage is attached to the hyosuspensorial ligament in approximately the position of Ridewood's interhyal, and three little bits of cartilage lie along the dorsal and anterior edges of the hyomandibula, attached to it by connective tissues.

The jugular groove is closed externally, throughout nearly its entire length, by a tough connective tissue membrane, the vein thus being entirely enclosed in the cranial wall. With this vein thus enclosed it would seem as if a hyomandibula could readily shift upward along the cranial wall from a position ventral to the vein to one dorsal to it, but there is no indication whatever that any cartilage has so shifted. The epibranchials of the first two branchial arches are in contact, by their dorsal ends, with the neurocranium ventral to the jugular groove, and are strongly bound to it by short longitudinally disposed ligaments that would seem to be the dorsal interarcual ligaments. In each of the first three arches I find, as van Wijhe did, a small cartilage which has strictly the position of a pharyngobranchial. Fürbringer ('04) did not find these cartilages, but he found a small cartilage that connected the dorsal ends of the epibranchials of the

third and fourth arches and accordingly had the position of an interarcual cartilage rather than that of a pharyngobranchial.

The nervus hyomandibularis facialis and the vena jugularis, in embryos of *Ceratodus*, always issue through the posterior opening of the trigemino-facialis chamber (Allis '14 c). The wide space, in the adult, between the two foramina for the nerve and vein, to which reference has just above been made, then indicates the extent to which the posterior portion of that chamber has been enlarged by the invading and enveloping growth of the cartilage of the region, and that edge of the suspensorium to which the hyomandibula is attached still represents the hind edge of the lateral wall of the chamber. The so-called hyomandibula thus lies not only at a considerable distance from the neurocranium but also definitely lateral to the vena jugularis, and hence not at all in the relation to that vein and the neurocranium which both the pharyngohyal and epihyal have in the Elasmobranchii.

In embryos of *Ceratodus* the cartilages in the dorsal half of the hyal arch have been described by Sewertzoff, Fürbringer, Krawetz, Edgeworth and Greil, and not only do the homologies proposed by these several authors for the several cartilages here developed differ greatly, but even the descriptions themselves differ, in certain respects, markedly from each other.

Sewertzoff ('02) found, in an embryo of *Ceratodus* somewhat older than Semon's Stage 47, a little cartilage which is said to lie immediately posterior to the nervus hyomandibularis facialis and to connect the ceratohyal with both the processus oticus palatoquadrati and the auditory capsule (mit dem Pr. oticus resp. mit der Ohrkapsel). The ventral end of this little cartilage is said to be attached to a ligament which is evidently the hyosuspensorial ligament of descriptions of the adult, while its upper end simply touches, without being attached to, the processus oticus palatoquadrati dorsal to the point of attachment of the ligament and dorsal also to the nervus hyomandibularis facialis. Sewertzoff considers this cartilage to be a rudimentary hyomandibula and the homologue of the cartilage so-named by Huxley in the adult. He did not find this cartilage in embryos of Stage 47, or younger. To facilitate the descriptions and avoid confusion it will hereafter be referred to, in brackets, as the cartilage *Ex*.

Fürbringer ('04) describes, in an embryo of Stage 48, a little cartilage that is said to lie above and anterior (oberhalb und oral) to the branchial skeleton and to come into contact, by its dorsal end, with the anterior portion of the auditory capsule. In younger stages it is said by Fürbringer not to have this contact. Fürbringer calls it the hyomandibula and says that he is convinced that it is the homologue of the cartilage so-named and described by Sewertzoff, notwithstanding that it is quite widely separated from the ceratohyal and hence does not connect that cartilage with the auditory capsule. He is undecided as to whether or not this so-called hyomandibula of embryos is the homologue of Huxley's hyomandibula of the adult, and he adds that this latter cartilage in no way differs from a branchial ray, as Pollard ('94) had previously maintained.

Krawetz ('10) says that the cartilages described by Sewertzoff and Fürbringer are totally different cartilages, for he finds them both in one and the same embryo. He retains the name hyomandibula for the cartilage described by Fürbringer, and it will hereafter be referred to, in brackets, as the cartilage *Ph*. Krawetz says that in Stages 45/46 and 46/47 this cartilage (*Ph*) is found imbedded in a strand of connective tissue extending from the antero-ventral wall of the auditory capsule to the 'processus palato-basalis quadrati,' this process quite certainly being, as I have recently shown (Allis '14 c), the processus basalis palatoquadrati and not the processus palatobasalis. The hyosuspensorial ligament is shown well developed in figures of embryos of these ages, but there is no ligamentous or connective tissue connection shown (figs. 10 a, 18) between this hyosuspensorial ligament and the one in which the so-called hyomandibula (*Ph*) is said to be imbedded. This latter ligament thus has, at this stage, no relation to the hyal arch except in that it has imbedded in it the little cartilage (*Ph*) said by Krawetz to represent the hyomandibula. The hyomandibula (*Ph*) is said to lie ventral (internal) to the nervus hyomandibularis facialis, and it is shown, in the figures, lying ventro-lateral to the vena jugularis and dorso-lateral to the arteria carotis (lateral dorsal aorta).

In a slightly older embryo of *Ceratodus*, Stage 47, Krawetz says that a connective tissue strand appears, connecting his hyo-

mandibula (*Ph*) with the hyosuspensorial ligament, and at the point where this strand joins the ligament, in intimate relation to the ligament, a hitherto undeveloped cartilage appears. This second cartilage, called by Krawetz the symplectic, is shown, as above described, in his figure 10 b, said to be of a model of an embryo of Stage 46/47, the cartilage there lying on the dorso-lateral surface of the two strands of ligamentous tissue at their point of junction, and in no way imbedded in them. In a slightly older embryo Krawetz shows, in transverse section (l. c., fig. 20), what is said to be the same cartilage, but it now lies directly between, and hence imbedded in the two ligamentous strands above described, at their point of junction. On the other side of the head of this specimen the hyomandibula (*Ph*) is said not to be present, and the symplectic, shown in sectional view in Krawetz's figures 21 and 22, is said to have here the position of the hyomandibula (*Ex*) of Sewertzoff's descriptions, but its dorsal end has not yet reached the processus oticus palatoquadrati. These two so-called symplectic cartilages, so markedly different on opposite sides of the head of the same specimen, are quite certainly identical, as Krawetz concludes, the cartilage evidently undergoing rapid development at about this Stage 47. As one of the two is also certainly identical with Sewertzoff's hyomandibula they will both be hereafter referred to, as that cartilage is, as the cartilage *Ex*.

In a slightly older embryo, Stage 47/48, Krawetz finds hyomandibular (*Ph*) and symplectic (*Ex*) cartilages on both sides of the head of the same embryo, and each symplectic cartilage (*Ex*) now reaches the related processus oticus palatoquadrati, exactly as the cartilage shown by Sewertzoff does. Krawetz (l. c., p. 359) concludes that the two cartilages *Ph* and *Ex* represent the entire dorsal half of the hyal arch, and as the cartilage *Ph* is considered by him to represent the hyomandibula the cartilage *Ex* becomes, doubtless by comparison with the conditions in the Teleostomi, the symplectic, and is said to represent the hyomandibula and symplectic combined of Huxley's descriptions of the adult. The antero-lateral end of the hyomandibula (*Ph*) is shown, at this stage, attached by ligament to the internal surface

of the palatoquadrate immediately posterior to the root of the processus basalis. The postero-mesial end of the hyomandibula has fused with the auditory capsule at the posterior edge of what Krawetz calls the foramen for the N. recurrens and R. hyomandibularis N. facialis, this foramen being the posterior opening of the trigemino-facialis chamber of my determinations (Allis '14 c). A little dorsal process of the hyomandibula (*Ph*) partly encloses, externally, the vena jugularis, and this little process will hereafter be referred to as the cartilage *Ia*. The connective tissue strand which connected the hyomandibula (*Ph*) and symplectic (*Ex*) in earlier stages is said to have now completely disappeared, and the hyomandibula (*Ph*) is here referred to (l. c., p. 357) as "ein neues Knorpelstück" although it is evidently the same cartilage as the similarly named one of earlier stages. It is said by Krawetz to be a serial homologue of the epibranchials of his descriptions; but it is to be especially noted that these so-called epibranchials (l. c., fig. 10 a) lie at an appreciable distance dorsal to the so-called ceratobranchials, the two sets of cartilages having apparently chondrified independently of each other instead of as a single piece which later segments, which is so frequently said to be the invariable rule, in all fishes, for the epal and ceratal elements of the branchial arches, and less frequently for the pharyngeal and hypal elements. These so-called epibranchials of Krawetz's descriptions may then be pharyngobranchials, and Greil's ('13) description of them, to be given later, seems to confirm this conclusion. The so-called epibranchial of the second branchial arch is said by Krawetz frequently to come into contact with the wall of the auditory capsule, separated from it by only a delicate epichondrial membrane, and in the adult, as already stated, I find the dorsal end of what is certainly the epibranchial of this arch, and also that of the first branchial arch, in contact with the neurocranium.

In embryos slightly older than Stage 48, the hyomandibula (*Ph*) is shown by Krawetz (l. c., fig. 6) extending entirely across the posterior opening of the trigemino-facialis chamber, and from the middle of this cartilaginous cross-bar the little dorsal process (*Ia*) of the preceding stage has extended dorsally and is said to

have fused with the ventro-mesial surface of the processus oticus palatoquadrati. The large posterior opening of the trigemino-facialis chamber is thus cut into three parts, and the rami recurrens and hyomandibularis facialis are said to both issue through the dorso-posterior one of the three. What the ramus recurrens is I can not make out either from Krawetz's or Greil's descriptions, but the important point in this discussion is that the hyomandibularis facialis issues posterior to the little dorsal process (*Ia*). This little process must accordingly be found in that part of the cranial wall of the adult that lies antero-lateral to the foramen faciale, between that foramen and the posterior edge of the suspensorium (see van Wijhe's figure), thus forming part of the cartilage that invades and fills up the trigemino-facialis chamber and not a part of the lateral wall of that chamber, as I concluded in a recent work (Allis '14 c).

Edgeworth ('11), who evidently had not seen Krawetz's earlier descriptions, says (p. 212) that in Stage 42 of embryos of *Ceratodus* "the hyoid bar extends upwards and inwards towards the under surface of the pro-cartilaginous tract connecting the parachordal plate with the auditory capsule, forming the hyomandibula, the original bar forming the ceratohyal and hypohyal. The upper part of the originally pro-cartilaginous hyomandibula chondrifies; the lower forms a fibrous tract connecting the outer end with the upper end of the ceratohyal. In Stage 48 a down-growth occurs from the outer edge of the auditory capsule, external to the hyomandibular branch of the VIIth, and becomes separated, forming a cartilage abutting against the outer end of the hyomandibula, and a second more dorsally situated piece is subsequently cut off from the auditory capsule." Edgeworth further says (l. c., p. 213) that probably no part of his 'true hyomandibula' is preserved in the adult, and that "The cartilage (or cartilages) cut off from the auditory capsule is probably, from its relation to the hyomandibular branch of the VIIth, that described by Huxley, Ridewood, Ruge, and Sewertzoff as the 'hyomandibula.'" The vena jugularis is shown by Edgeworth (fig. 50, p. 223) lying dorsal to his hyomandibula and internal to the one or two little cartilages said to have been cut off from the auditory capsule.

The hyomandibula of these descriptions of Edgeworth's is the similarly named cartilage (*Ph*) of both Fürbringer and Krawetz. The ventro-lateral one of the two pieces said to be cut off from the auditory capsule is certainly the symplectic (*Ex*) of Krawetz and the dorso-mesial one the dorsal process (*Ia*) of the hyomandibula (*Ph*) of the latter author's descriptions. That one of these two authors should consider these two cartilages to have been cut off from the auditory capsule while the other considers them to have been developed wholly independent of the capsule, is evidently simply a difference of interpretation of the same phenomena. Edgeworth says that the two cartilages lie 'external' to the nervus hyomandibularis facialis, without saying whether the nerve issues anterior or posterior to them. But, as he compares one or both of the cartilages with the hyomandibula of Huxley's descriptions of the adult, it would seem that one of them, at least, and probably both, were considered by him to lie posterior to the nerve. This agrees with what both Sewertzoff and Krawetz say of the cartilage (*Ex*) but is the exact opposite of what Krawetz says of his little dorsal process (*Ia*).

Greil ('13), in the latest work published on this subject, makes no reference to either Krawetz's or Edgeworth's earlier works, and hence had probably not seen them when his own was sent to press. In an embryo of Stage 47 he describes and figures (l. c., p. 1293) a small cartilage, lying internal to the nervus hyomandibularis facialis, which he calls the epihyal or hyomandibula. This little cartilage is the hyomandibula (*Ph*) of Fürbringer, Krawetz and Edgeworth, and is said by Greil, as it was by Krawetz, to be a serial homologue of the epibranchials. These latter cartilages are said by Greil (p. 1239) to be directed postero-mesially from the dorsal ends of the ceratobranchials, instead of antero-mesially as the epibranchials invariably are in all other gnathostome fishes, and as the dorsal ends of the ceratobranchials are shown (fig. 23, pl. 61) curving dorso-antero-mesially in the usual position of an epibranchial, comparison with my descriptions of the adult seems to me to conclusively show that the so-called epibranchials of both Greil's and Krawetz's descriptions are pharyngobranchials, and that the epibranchials have

not yet been segmented off from the dorsal ends of the ceratobranchials. The pharyngobranchials, as thus determined, lie anterior to the efferent arteries of their respective arches, instead of posterior, as in the several Plagiostomi that I have examined in connection with this work, and they each articulate with the dorsal end of the related epibranchial instead of with the posterior edge of that end, as in the Plagiostomi; *Ceratodus* possibly representing, in this respect, an intermediate stage between the plagiostome and teleostome conditions.

In an embryo of Stage 48, Greil shows (l. c., fig. 543, p. 1397) two of the so-called epihyal or hyomandibular cartilages, and he says that there may even be three of them. The two cartilages shown in his figure 543 form a curved line which extends from beneath the auditory capsule nearly to the dorsal end of the ceratohyal; and the nervus hyomandibularis facialis runs outward and downward immediately external to them both, this doubtless being the relation of the nerve to the third cartilage also, when present. The dorsal one of these two or three cartilages is certainly the hyomandibula (*Ph*) of Greil's descriptions of earlier stages, and it does not reach, by an appreciable distance, the wall of the auditory capsule, this embryo of Stage 48 thus being less advanced in this respect than Krawetz's embryo of Stage 47/48. The vena jugularis lies dorsal to the dorsal end of the cartilage, and the arteria carotis interna (lateral dorsal aorta) ventro-mesial to it. The second cartilage shown in Greil's figure corresponds closely to the symplectic (*Ex*) of Krawetz's figure 20, of an embryo slightly older than Stage 47, and is quite certainly that cartilage notwithstanding that it does not, in this embryo of Stage 48, extend upward to the processus oticus palatoquadrati; Greil's embryo here again representing a less advanced stage of development than Krawetz's younger embryos. The third cartilage may be called the cartilage *Eh*. These two or three little cartilages are said to form the entire dorsal half of the cartilaginous bar of the hyal arch, and, as Greil calls them all epihyal or hyomandibular cartilages, it seems certain that the term epihyal is used in the sense of epi-pharyngohyal and that he considered the several cartilages to represent, together, the se-

lachian hyomandibula. No other cartilage related to the dorsal end of the hyal arch is described by Greil.

From these several descriptions of embryos of *Ceratodus* it is certain that three, and practically certain that four, separate and independent cartilages may develop in the dorsal half of the hyal arch of *Ceratodus*, and as this corresponds exactly with the number found in the dorsal half of the arch in the Batoidei there is certainly no reason to assume, either that the epihyal has here been broken up into several pieces, or that an epi-pharyngohyal has been broken up into any others than the two normal pieces. The cartilage *Ph* has exactly the relations to the auditory capsule, the vena jugularis, the arteria carotis interna (lateral dorsal aorta) and the nervus hyomandibularis facialis that the hyomandibula of the Batoidei has to the same structures. As in those fishes, its lateral end is attached to the palatoquadrate, by ligament in young embryos but by fusion in older ones, and it lies dorsal to the dorsal end of the mandibular aortic arch, which is probably the relation of the hyomandibula of the Batoidei to that artery, but I could not control this in my two already partly dissected specimens of the latter fishes.

This cartilage *Ph* is thus certainly a pharyngohyal, and, together with its dorsal process, can properly be called the hyomandibula. The dorsal process (*Ia*) has the same relations to the pharyngohyal, the vena jugularis and the nervus hyomandibularis facialis that the hook-like process of the hyomandibula of *Torpedo* has, and is quite certainly the homologue of that process and hence of the corresponding, independent cartilage of *Narcine*. It is accordingly quite certainly an interarcual cartilage. The cartilage *Ex* lies lateral to the vena jugularis and posterior to the nervus hyomandibularis facialis, and, in Edgeworth's figure 20, its ventral end is in contact with the lateral (distal) end of the pharyngohyal (*Ph*). In the adult it has been pulled away from the pharyngohyal by the great expansion of the trigemino-facialis chamber, but it is still attached, by its ventral end, to the hyosuspensorial ligament dorsal to the cartilage *Ex*. It has accordingly all the relations to the other structures of the hyal arch, so far as they are given, of an extrabran-

chial of the arch, and its relatively late and sudden development are also in accord with its being that element. That it can be the homologue of the symplectic of the Teleostomi, to be later considered, seems quite improbable. The only remaining cartilage, *Eh*, is then the epihyal, and it has all the relations to the adjacent structures of such an element. All the cartilages in this arch of *Ceratodus* are thus those normally found in a visceral arch and they could all have been readily derived from a condition resembling but preceding that actually found in the *Torpedinidae*.

The Teleostomi can now be considered; and here, as with the *Elasmobranchii* and *Dipneusti*, the descriptions of the development of the hyal cartilages are conflicting. For the purposes of this paper it will suffice to refer only to Edgeworth's somewhat recent work.

Edgeworth says ('11, p. 207) that in 8 mm. embryos of *Acipenser* the hyal bar is still in a procartilaginous and unsegmented condition and does not extend up to the auditory capsule. The *nervus hyomandibularis facialis* is said to pass "over the upper end of the bar and then downwards outside it," which would seem to be simply the relation that the branchial nerves of the adult have to the cartilaginous bars of the related branchial arches. In $8\frac{1}{2}$ mm. embryos of this fish the hyal bar is said to extend upward in front of 'and outside' the *nervus hyomandibularis facialis*, and the definitive hyomandibula is said to be formed from this upgrowth together with the upper portion of the primitive bar, when that bar later segments. The hyomandibula is thus here said to develop exactly as it is claimed by Edgeworth (l. c., p. 206) to develop in *Scyllium*, differing only in that the upgrowth of the hyal bar toward the auditory capsule is said to pass anterior to the *nervus facialis* in *Acipenser* while no mention is made of the relations of the upgrowth to the nerve in *Scyllium*. No mention is made by Edgeworth either of a symplectic or an interarcual cartilage.

In *Polypterus* the hyomandibula is said by Edgeworth to develop exactly as in *Acipenser*, no account apparently being taken of the important fact that the relations of the hyomandibula to the

hyoideus and mandibularis branches of the nervus facialis are not the same in the adults of the two fishes (van Wijhe '82).

In all the other teleostoman embryos that Edgeworth examined, the sequence of events is said by him (l. c., p. 209) to be the same as in *Acipenser*, but, in all these other teleostomans, "the VIIth nerve, at first winding round the hyoid bar, subsequently pierces the hyomandibula owing to chondrification spreading round it; the more primitive condition is preserved in *Acipenser* and *Polypterus*." Here again the fact that the ramus mandibularis facialis runs outward, anterior to the hyomandibula, in *Polypterus*, is apparently overlooked, for if it issued primarily posterior to that cartilage, as it does in *Acipenser*, it would evidently have to cut entirely through it in order to acquire the position it has in the adult. Edgeworth says (p. 207) that, according to Rutherford ('09), "in the brown trout a downgrowth of no great size, from the periotic capsule at the edge of the foramen ovalis, joins with the symplecticum in front of the VIIth nerve, and finally unites with the primitive hyomandibula." Edgeworth says that in no case did he himself find any such downgrowth from the periotic capsule, which rather strikingly recalls the fact that he himself describes a downgrowth from the periotic capsule in *Ceratodus* which corresponds to what Kraetz describes as an upgrowth from the hyal bar in the same fish; and here, as there, it seems probable that the two observers simply differently interpreted the same phenomena, and that both recorded growths simply represent the chondrification of an independent cartilaginous element which preëxisted, in situ, in tissues that were not sufficiently differentiated in the sections of earlier stages to be recognizable.

If the development of the hyomandibula of the Teleostei, as here described by Edgeworth, be compared both with the conditions found in embryos of *Ceratodus* and those in the adult of *Torpedo*, it seems certain that the so-called upgrowth of the hyal bar said by Edgeworth to pass anterior to the nervus hyomandibularis facialis is the homologue of the hook-like process of the hyomandibula (pharyngohyal) of the adult *Torpedo*, and hence also of the dorsal process of the hyomandibula (pharyngohyal)

of embryos of *Ceratodus*, and that the later development of cartilage said by Edgeworth to spread round and enclose the *nervus hyomandibularis facialis* is simply the chondrification, in situ, of the extrabranchial of the arch, and its concomitant fusion with the pharyngohyal. The anterior articular head of the teleostean hyomandibula is then, as are probably the corresponding cartilages in *Torpedo* and *Ceratodus*, an interarcual cartilage which has fused with the pharyngeal element of the hyal arch, and the posterior articular head is the extrabranchial of the arch fused with the same element; and, because of the much more advantageous articulation thus acquired, the primitive dorsal end of the pharyngeal element has lost, or never acquired, direct contact with the neurocranium. The interhyal (stylohyal) so closely resembles the epihyal of *Ceratodus* and the Batoidei that it is quite unquestionably that element, and this is in accord with Stöhr's ('82) statement that it is segmented from the dorsal end of the ceratohyal after that element has separated from the hyomandibula, exactly as it is said by Gegenbaur to be segmented from that element in the Batoidei.

This thus accounts, in the Teleostei, for all the independent cartilaginous elements that take part in the formation of this dorsal half of the inner cartilaginous bar of the hyal arch in the Elasmobranchii and Dipneusti, and, if the symplectic be not simply a ventral process of the pharyngohyal, similar to the one so named by Gegenbaur in the Batoidei, it must be looked for in other cartilages of the region, and it would seem as if it must be a specially modified branchial ray of the mandibular arch. That it is an entirely new element here added to the hyal arch seems wholly improbable.

When the hyomandibular visceral cleft began to undergo reduction it is probable that it was first pinched off and closed at the middle of its length, for remnants or rudiments of the branchiae of the cleft have persisted both dorsal and ventral to this point—dorsally as the spiracular branchiae and ventrally as the thyreoid. It is also natural that the cleft should here have been first closed, for, as is actually the case in the branchial arches of living fishes, the dorsal and ventral ends of the inner

cartilaginous arches were undoubtedly in a measure held and fixed at a certain distance from each other, while at the middle of their lengths, where the dorsal and ventral halves articulated with each other, a certain freedom of motion existed. Furthermore, while it is the dorsal portion of the cleft that longest persists in fishes, it is said to be the ventral portion of the cleft that persists the longer in the Amphibia. But even if the cleft were not actually closed here first, it is here that the branchial rays first differentiate in ontogeny (Dohrn), and here that they are the most strongly developed. It is accordingly natural to suppose that, as the cleft aborted, whether this began at the middle of its length or at its ventral end, a time would come when these middle rays of the mandibular series, or their protons (Anlagen), developing in the tissues which bridged the space between the mandibular and hyal arches, would come into contact, by their distal ends, with the hyal cartilages. These rays, or their protons, would then give rise, not only to the symplectic cartilage of the Teleostei, but also to the several and varying ligaments that here connect the mandibular and hyal arches in the Selachii, excepting probably the large ligamentum mandibulo-hyoideum, which is said by Edgeworth ('11, p. 212) to have been derived from the muscles of the region. In the Batoidei the only one of these several ligaments that is shown by either Parker or Gadow is, as already stated, a stout one extending from the ventral end of the quadrate to the ventral end of the hyomandibula, which is exactly the position of the symplectic cartilage in the Teleostei. The other ligaments here found in the Selachii have perhaps not been developed in the Batoidei because, in these fishes, the space separating the mandibular and hyal arches was too wide to be bridged by the tissues that represented the primitive rays, these tissues then here aborting or being dispersed.

The symplectic of the Teleostei is said by Stöhr ('82) to be developed as a primarily independent element which lies close against the hinder edge of the quadrate and later fuses with a ventral process of the hyomandibula; which is strictly in accord with its being a branchial ray related to the mandibular arch. It lies, in the adult, internal to the arteria hyoidea (afferent man-

dibular artery), which is exactly the relation that a mandibular ray would normally have to the afferent artery of its arch; and, if it be a mandibular ray, the apparently tortuous course of the arteria hyoidea would be explained, for, without other apparent reason, that artery passes from the internal surface of the so-called palatine arcade outward across the ventro-posterior edge of the symplectic, crosses the external surface of that element, and then runs inward across its dorso-anterior edge to reach the pseudobranch. The relations of the rami mandibularis externus and internus facialis to the symplectic would also receive explanation, for the attachment of the symplectic to the hyomandibula is always, in all the Teleostei with which I am familiar, ventral to the point where these two nerves separate from each other, and, as one of the two nerves runs outward and the other inward, the symplectic, if it be a mandibular branchial ray, would naturally lie either between them or internal to them both, according to the position of the point of contact of the outer end of the ray with the hyomandibula. Furthermore, the symplectic is said to be found, frequently in the Teleostei, completely and indistinguishably fused with the hind end of the quadrate, which would be wholly natural for a branchial ray related to that cartilage. The little chain of cartilages which, in *Torpedo*, connects the hyomandibula with the symplectic cartilage, would then be derived from other rays of the mandibular series, for, that these cartilages can represent the symplectic, seems improbable from their relations to the afferent mandibular artery. This artery, in the Teleostei, crosses the external surface of the symplectic, as just above stated, while in the Batoidei it would seem as if it must lie internal to the little chain of cartilages, for it is well known that it lies postero-internal to the spiracular cartilage (Dohrn '85).

As in the Batoidei, and apparently for the same reason, there is, in the Teleostei, no inferior postspiracular ligament. That the teleostean *M. adductor hyomandibularis*, which has so markedly the position of the selachian inferior postspiracular ligament, can be the homologue of that ligament seems improbable because of the relation of the one to a pharyngeal and of the other to an

epal element of the arch, and also because of the difference in the manner of innervation of the adductor hyomandibularis and the interarcuales dorsales II of the branchial arches, from the hyal member of which latter muscles the inferior ligament of the Selachii is apparently derived.

The hyomandibula of the Teleostei is thus seen, if I am correct in my conclusions, to have assimilated interarcual and extrabranchial cartilages related to the hyal arch and by that means to have acquired articulation with the auditory capsule dorsal to the vena jugularis and in protective relation to it. If these interarcual and extrabranchial components of the teleostean hyomandibula were to fuse with the auditory capsule at the places where they actually articulate with it, as the interarcual cartilage actually does in *Ceratodus*, and, if the primitive dorsal end of the pharyngeal component were also to fuse with that capsule ventral to the vein, as it also actually does in *Ceratodus*, conditions would arise in the hyal arch of the Teleostei which would seem to be exactly similar to those actually found in the mandibular arch of *Ceratodus* (Allis '14 c); the anterior articular head of the teleostean hyomandibula being the serial homologue of the processus ascendens palatoquadrati of *Ceratodus*, the posterior articular head being the serial homologue of the processus oticus palatoquadrati, and the pharyngeal element of the hyomandibula the serial homologue of the processus basalis palatoquadrati. The correspondence is in every way too exact to leave any doubt of this, in so far, at least, as the last two processes are concerned. The processus ascendens might equally well be, so far as its relations to the nerves and blood vessels are concerned, the extrabranchial of the premandibular arch, and, furthermore, there seems here no reason, as in the hyal arch, for an interarcual cartilage to have acquired contact with the cranial wall dorsal to the vena jugularis. But however this may be, the derivation of these three processes of the palatoquadrate from elements that are, in the branchial arches of the adults of living fishes, found either as wholly independent cartilages or fused one with the other or with the epal element of their arches, explains how they can be, in certain fishes (*Ceratodus*), fused with both the

palatoquadrate and the cranial wall; in certain other fishes (Holo-
lostei, Teleostei) fused only with the cranial wall; and in embryos
of the Amphibia fused only with the palatoquadrate.

In the Plagiostomi the pharyngeal element of the mandibular
arch, as an independent cartilaginous element, does not exist,
and the tissues representing it have probably been dispersed and
utilized to form adjacent portions of the neurocranium (Allis
'14 b). It may perhaps be in part represented in the low ridge
described by me (Allis '14 a) on the dorsal edge of the palatoquad-
rate of *Chlamydoselachus* and there considered by me to be the
homologue of the processus basalis palatoquadrati of the Am-
phibia; but it seems to me much more probable that this ridge
simply represents the dorsal edge of the quadrate, which is the
epal element of the arch. The low ridge in *Chlamydoselachus*
can nevertheless properly be called the processus basalis, for
even in the Amphibia this process may, as in *Rana fusca* (Gaupp),
develop in part from cells related to the quadrate and in part
from cells wholly independent of that cartilage, these latter cells
evidently representing the pharyngeal element of the arch.

The extrabran-
chial of the mandibular arch has, in contradis-
tinction to the pharyngeal element, quite certainly been pre-
served, in the Plagiostomi, in the frequently largely developed so-
called spiracular cartilage of the Batoidei, as is evident from a
consideration of Gegenbaur's ('72) and Parker's ('76) several
figures of these fishes. This spiracular cartilage of the Batoidei
lies definitely lateral to the vena jugularis; its ventral end is at-
tached, either by ligament or by a chain of cartilaginous rods, to
both the palatoquadrate and the anterior edge of the hyoman-
dibula; its dorsal end is either attached by ligament to the cran-
ial wall dorsal, to the vena jugularis (*Raia radiata*, Allis), or ar-
ticulates there with that wall (*Raia clavata*, Parker); and the
nervus hyomandibularis facialis issues posterior, and the nervus
trigeminus anterior to it.

Dohrn ('85) thought this spiracular cartilage of the Batoidei
could not have been derived from a branchial ray or rays of the
mandibular arch, and one of his reasons in support of this view is
that the spiracular cartilage lies anterior to the entire blood-ves-

sel apparatus of the spiracular gill while the branchial rays of the hyal and branchial arches always lie posterior to the artery of the related arch. This statement, which is doubtless true for conditions found in embryos, with which I am not familiar, is not true for those found in the adult. In the adult *Mustelus* I find the afferent branchial arteries lying anterior to the branchial rays, but the two efferent arteries of each branchial arch lie, one anterior and the other posterior to those rays. In the hyal arch the single efferent artery lies posterior to the rays, and this would naturally be the position of the single artery in the mandibular arch if that part of this artery which is related to the spiracular gill is developed as are the corresponding arteries in the posterior arches. The spiracular cartilage, derived from the dorsal ray of the mandibular series, would then be in proper relation to the efferent artery of that arch, while the symplectic cartilage of the Teleostei, derived from one or more of the middle rays of the series, and lying ventral to the persisting gill, would be in proper relation to the afferent artery.

Gegenbaur says ('72, p. 202) that both Henle and Müller called the spiracular cartilage of the Torpedinidae the cartilago pterygoidea, and compared it with what was later described by Huxley in the Teleostei as the metapterygoid. Parker ('76) also calls the cartilage in *Raia* the metapterygoid, and says (l. c., p. 219) that it "answers to the otic process of an Amphibian;" and Huxley ('76) says the same of the spiracular cartilage of *Cestracion*. My conclusions, if correct, thus confirm these earlier determinations in so far as the homology of the spiracular cartilage of the Batoidei with the otic process of the Amphibia is concerned, but as this otic process is represented, in teleosts, in a part of the lateral wall of the trigemino-facialis chamber, it is not the homologue of the metapterygoid of those fishes, nor is it the homologue of a part of the palatoquadrate of selachians, as Huxley concluded.

The several cartilages that have been described as spiracular cartilages in different selachians are certainly not all of them homologues of the spiracular cartilage of the Batoidei. This is

espécialement true of these cartilages in *Chlamydoselachus*, where, in the one specimen of this fish that I have examined, I find three in place of the one described by Fürbringer ('03). These cartilages of *Chlamydoselachus* are quite certainly rudiments of the ordinary branchial rays of the mandibular arch, and hence not rudiments of the dorsal extrabranchial of that arch. They may, however, represent the little chain of cartilages that, in *Torpedo*, connects the spiracular cartilage of that fish with the hyomandibula. This whole subject needs further investigation, which my material does not at present permit.

The eye-stalk, under my present interpretation of the mandibular cartilages, can not be the homologue of the processus ascendens palatoquadrati of amphibians, as I have quite recently suggested (Allis '14 a). It still, however, seems to me that it must be a cartilage related to the dorsal half of the premandibular arch, and its posteriorly directed position, and its contact with the ventral portion of the cranial wall ventral to the orbital sinus both seem to indicate that it is a pharyngeal element of its arch. It lies ventral to the nervus ophthalmicus profundus, which is its proper relation to that nerve if both it and the nerve belong to the premandibular arch; but its relations to the efferent pseudobranchial artery and to the superior and inferior divisions of the nervus oculomotorius need explanation. Luther ('09) has described two little muscles in *Stegostoma* which are said to arise from the cranial wall and, running forward, to be inserted on the eye stalk, which, if the stalk is a premandibular pharyngeal element, would strongly suggest interarcuales dorsales muscles related to that arch.

In the Chondrostei the hyomandibula articulates with the neurocranium by a single articular head, and this head apparently has exactly the relations to the vena jugularis and nervus hyomandibularis facialis that the anterior articular head of the teleostean hyomandibula has. The conditions, however, differ in that, in *Polyodon*, and hence probably in the other Chondrostei also, the vena jugularis and nervus hyomandibularis facialis traverse a canal in the cranial wall which is apparently not a trigemino-facialis chamber comparable to the one found in the

Holostei and Teleostei (Allis '11 a, p. 292); and in that the hyomandibula articulates, in *Polyodon*, in part with the lateral wall of that canal. There is, in the Chondrostei, not the slightest indication of even a rudiment, of a postfacialis articular head of the hyomandibula, the extrabranchial of the arch thus not so being accounted for. There is an interhyal, which is quite certainly the strict homologue of that found in the Teleostei. The symplectic is a large and independent cartilage which extends from the ventral (distal) end of the hyomandibula to the articular end of the palatoquadrate, strongly attached to both those cartilages by ligaments or ligamentous tissues, and, as in the Teleostei, it would seem as if it must have been developed from one or more of the middle branchial rays of the mandibular series. Its relations to the arteria hyoidea are the same as those of the symplectic process of the Teleostei (Allis '11 a, p. 260), but its relations to the nervus facialis would seem, from van Wijhe's ('82) figures and descriptions, to differ, in both *Acipenser* and *Polyodon*, from those in the Teleostei, and even to differ from each other in *Acipenser* and *Polyodon*.

In *Polyodon* I find the ramus mandibularis internus facialis traversing a little notch in the hind edge of the symplectic which is also traversed by the efferent hyoidean artery (Allis '11a, p. 286), the nerve thus lying external to the cartilage while dorsal to the notch, but internal to the cartilage when ventral to the notch, the notch thus appearing to represent the interval between two rays that have here fused to form the symplectic. Parker ('82) considered the symplectic of *Acipenser* to be a piece segmented from the ventral end of the epihyal, the remainder of the latter element forming the hyomandibula and the pharyngohyal being wholly wanting. But this breaking up of any one of the four typical elements of a visceral arch into two or more pieces, and their perpetuation by inheritance, finds no support whatever in my work.

In *Acipenser*, van Wijhe ('82) shows a small process projecting upward on the lateral surface of the hyomandibula between the mandibularis and hyoideus branches of the nervus hyoman-

dibularis facialis. Parker ('82) does not show this process in *Acipenser* nor does Bridge ('79) show it in *Polyodon*, but Bridge found a nodule of cartilage in *Acipenser* and a filament of cartilage in *Polyodon*, both of which are said to lie in a groove on the lateral surface of the hyomandibula and which may accordingly represent the process of van Wijhe's descriptions. In a single specimen of *Polyodon* that I have rather summarily examined I find the process on the mesial, instead of the lateral, surface of the hyomandibula, and it lies, as the process does in *Acipenser*, between the two branches of the mandibularis facialis. Bridge considered the little cartilage found by him to be a remnant of a branchial ray, and as he does not specify to which arch it belongs, it was undoubtedly considered by him to belong to the hyal arch. But if the little cartilage is the homologue of the process described by van Wijhe it would seem as if it could not be a branchial ray of the hyal arch, for the process lies anterior to the ramus hyoid-eus facialis instead of posterior to it, as it normally should if it were a ray of the related arch. Its relations to the afferent artery of the arch are not given, but it would seem as if it must lie anterior also to that artery, which would not be normal. It certainly lies dorso-posterior to both the afferent and efferent mandibular arteries. It may accordingly be the extrabranchial of the mandibular arch; and as there seems to be no trigemino-facialis chamber in this fish, that element of the mandibular arch would be thus accounted for. Bridge describes, in *Polyodon*, a ligament which he calls the metapterygoid ligament and which he says extends from near the dorsal end of the hyomandibula to "the smaller of the two parasphenoidal alae," passing upward and forward ventral to the spiracular canal. This ligament is said to support, on its spiracular surface, the short branchial filaments of the mandibular gill, and it hence corresponds, in position, to the spiracular cartilage and the related ligament and chain of little cartilages in *Torpedo*. There is, however, no spiracular cartilage related to it.

In *Polypterus* the hyomandibula differs in certain important respects from that both of the Chondrostei and Teleostei, and, as in the case of the Chondrostei, the conditions here need further

investigation. The rami hyoideus and mandibularis facialis here run outward on opposite sides of the hyomandibula, the one posterior and the other anterior to it, this being exactly the relations that these two nerves have to the little process on the lateral surface of the hyomandibula of *Acipenser*. If that little process were to undergo marked development, and the actual articular head of the hyomandibula to undergo a corresponding reduction, the hyomandibula of *Polypterus* would apparently arise.

In vertebrates higher than fishes I have made but little attempt to trace the hyomandibula, for it is evident that, as the descent of these higher vertebrates is not known, any one, or even all, of the forms of hyomandibula developed in fishes might also be there found; and to determine which one of them is represented in any particular case requires an intimate knowledge of the anatomy and development of the region which I do not possess, and which, as in the case of fishes, the literature alone, of the subject would probably not give.

In Kingsbury and Reed's ('09) very careful descriptions of this region in the Urodela, the columella is said to develop from a group of cells which is shown, in a transverse sectional view of a 13-14 mm. embryo of *Amblystoma punctata*, lying close against the lateral wall of the vena petroso-lateralis (jugularis) and extending both dorsal and ventral to that vein, the ventral cells lying close against the lateral wall of the auditory capsule in the interval between the vena petroso-lateralis dorsally and the arteria carotis interna (lateral dorsal aorta) ventrally. Kingsbury and Reed say (l. c., p. 555) that "The derivation of these cells was not definitely determined. Younger embryos lend some support to the view that they migrate down around the vein." The basal, or fenestral plate of the columella is said to develop in that part of the mass of cells which lies ventral to the vena petroso-lateralis and is there in contact with the membrane that fills the fenestra vestibuli, these cells thus having the relation to the auditory capsule, to the vena petroso-lateralis and also to the arteria carotis interna, that the pharyngohyal has in *Ceratodus* and the Batoidei. The stylus of the columella develops in that part of the mass of cells that lies lateral to the vena

petroso-lateralis, and its distal end, which is said to be primarily connected with the 'squamosum,' secondarily has that connection transferred to the underlying palatoquadrate. These cells thus have the relation to the vein that either the extrabranchial or the interarcual cartilage may have to the vein in the hyal arch of fishes, and to determine which one of them is represented the relations of the stylus to the nerve, artery and vein of the arch should be known. This relation of the stylus to the nerve is given by Kingsbury and Reed, and in this respect the Urodela examined by them present two distinctly different groups. In the larger group the nervus hyomandibularis facialis is said (l. c., p. 610) to lie ventral and anterior ('cephalad') to the stylus, but in *Necturus*, *Proteus* and *Typhlomolge* the two branches of the nerve straddle the stylus, the ramus mandibularis lying ventral and anterior to it and the ramus jugularis (hyoideus) dorsal and posterior to it.

In both of these two groups of the Amphibia the basal plate of the columella is quite certainly, as just above stated, the pharyngeal element of the hyal arch. In the first mentioned group the stylus has the relations to the nervus hyomandibularis facialis that the posterior articular head of the teleostean hyomandibula has, and is probably the extrabranchial of the hyal arch. In the second group the stylus has the relations to the nerve that the hyomandibula of *Polypterus* has, and it is accordingly uncertain what element of the arch it represents. In the frog, the plectrum, which is said by Kingsbury and Reed to be the homologue of the columella of the Urodela, is said to lie below and anterior to the entire nervus hyomandibularis facialis, and accordingly has the relations to that nerve that the dorsal process of the hyomandibula of *Ceratodus* has, and is probably the interarcual cartilage that lies between the hyal and mandibular arches. It accordingly seems quite certain that the columellae in these three groups of the Amphibia are not homologous structures, and this offers a much more plausible explanation of the differing relations of the nervus hyomandibularis to the columella than the one usually given that the nerve has radically changed, for some unknown reason, its relations to that skeletal

structure. Krawetz concluded ('10, p. 360) that the stylus of all of the Urodela was represented in the strand of connective tissue that extends, in *Ceratodus*, from the anterior wall of the auditory capsule to the processus basalis palatoquadrati, but this strand of tissue lies ventral to the entire nerve instead of dorsal to, or straddling it.

In the Sauria the columella is said to lie (Gaupp '99, p. 1105, Kingsley '00, p. 218) between the chorda tympani (mandibularis internus) and the ramus hyomandibularis facialis, posterior to the former and anterior to the latter. The columella accordingly has the relations to these two nerves that the hyomandibula of *Polypterus* has. The extracolumella of these animals is said by Kingsley to be a primarily independent element, which later fuses completely with the stapes, and he is strongly inclined to consider it "as the remains of a visceral arch which has almost completely disappeared from between the hyoid and the mandibular arches," and the homologue, possibly, of the spiracular cartilage of the *Plagiostomi* (l. c., pp. 232-235). In a figure (l. c., fig. 2) said to be of an early stage of the lizard, the hyal cartilages are all shown, and the stapes as there shown is evidently, if it lies ventral to the vena petroso-lateralis as it does in the Urodela, an epipharyngohyal and not simply a pharyngohyal, for it is continuous ventrally with the ceratohyal. The extracolumella has strikingly the position of the symplectic of the *Chondrostei*, and it would seem as if it must be the homologue of that cartilage rather than of the spiracular cartilage of the *Plagiostomi*, as Kingsley suggests. The relations of the veins, arteries and nerves of the region would doubtless determine this.

The dissections of the several fishes examined in this work have all been prepared by my assistants, Mr. Jujiro Nomura and Mr. John Henry, Mr. Nomura's work being limited to *Chlamydoselachus*. The preparation and examination of the microscopical sections has all been done by Mr. Henry.

SUMMARY

In all fishes, so far as I can find from the literature and material at my disposal, the pharyngeal elements of the branchial arches always lie ventral to the vena jugularis and also primarily ventral to the dorsal aorta. But whenever these pharyngeal elements or the epal elements of the branchial arches come into contact with the neurocranium or the vertebral column, the point of contact, although still remaining ventral to the vena jugularis is then always lateral, and hence actually dorsal, to the dorsal aorta or its anterior prolongations, the lateral dorsal aorta of either side.

In all the Elasmobranchii, whenever the primary dorsal ends of the cartilaginous bars of the prebranchial arches come into contact with the neurocranium the point of contact is also always ventral to the vena jugularis and dorsal to the lateral dorsal aorta; and this is apparently also the relation of these blood vessels to the corresponding arches in *Ammocoetes*.

This would accordingly seem to be such a fundamental characteristic of the visceral arches of all craniate fishes that it is a legitimate conclusion that the dorsal ends of the inner cartilaginous bars of all of these arches, in all vertebrates, primarily lay ventral to the homologue of the vena jugularis of fishes, and that, when parts of the definitive cartilaginous bars of any of the visceral arches of any vertebrate articulate or fuse with the neurocranium dorsal to the homologue of that vein, those parts are primarily independent cartilages that have fused with the inner cartilaginous bars; or if it be that they are specially developed processes of those bars, those processes do not represent the primary dorsal ends of either the pharyngeal or epal elements of the inner bars.

Dorsal and ventral so-called extrabranchial cartilages are found more or less developed in the branchial and hyal arches in all of the Plagiostomi, and their bases have acquired protective relations to the related vena jugularis. In fishes other than the Plagiostomi these extrabranchial cartilages have never been described as such, so far as I can find, but the dorsal extrabranchials

are quite certainly represented in the suprapharyngobranchials of van Wijhe's descriptions of ganoids and *Polypterus* and the ventral ones in processes of certain of the hypobranchials of certain of those same fishes. The suprapharyngobranchials may be found as independent cartilages, or they may be found fused with the pharyngobranchial or epibranchial of their respective arches and so appearing as processes of those elements.

Interarcual cartilages, developed in or in relation to the dorsal interarcual ligaments and corresponding in position to the epitemal longitudinal branchial bars of the *Cyclostomata*, are found in many of the *Plagiostomi*, and, like the suprapharyngobranchials, they may be found either as independent cartilages or fused with the adjacent elements of the inner cartilaginous bars. Similar cartilages are apparently also found in *Scomber* and *Ceratodus*.

In the *Selachii* the hyomandibula is formed by the epihyal, in the *Batoidei* by the pharyngohyal, and in all these fishes the hyomandibula always articulates with the neurocranium ventral to the vena jugularis. In what is apparently definite correlation to this, the dorsal extrabranchial of the hyal arch persists, as an independent cartilage, in most if not in all these fishes. The interarcual cartilage that lies between the mandibular and hyal arches may be wholly wanting, may apparently be found as an independent cartilage (*Narcine*), or found fused with the anterior edge of the hyomandibula (*Torpedo*) and appearing as a process of that element.

In the *Holostei* and *Teleostei* the hyomandibula always articulates with the neurocranium dorsal to the vena jugularis, and hence is in protective relations to that vein; and quite certainly in definite correlation to this, there is no independent extrabranchial cartilage in the hyal arch and no superior postspiracular ligament or its related interarcual cartilage. These two cartilages, the extrabranchial and interarcual, are accordingly, in all probability, respectively represented in the posterior and anterior articular heads of the hyomandibula of these fishes. The interhyal is the epal element of the arch. The symplectic is probably a primarily independent cartilage, and is possibly a

hypertrophied middle one or ones of the branchial rays of the mandibular arch.

In the Chondrostei, the single dorsal articular head of the hyomandibula apparently corresponds to the anterior articular head (prefacialis portion) of the teleostean hyomandibula, and the interhyal to the interhyal of those fishes. The extrabranchial of the hyal arch is apparently wholly wanting, but the extrabranchial of the mandibular arch may possibly be represented in a little process, or a small and independent cartilage, found on the external surface of the hyomandibula. The symplectic is probably homologous with that of the Teleostei.

In the Dipneusti (*Ceratodus*) the hyomandibula, as properly identified in recent investigations, is formed by the fusion of an interarcual cartilage with the pharyngohyal, and accordingly corresponds to the anterior articular head (prefacialis portion) of the teleostean hyomandibula. The hyomandibula of Huxley's descriptions of the adult is probably the extrabranchial of the hyal arch, and the interhyal of Ridewood's descriptions the epihyal.

In the mandibular arch, the extrabranchial is found either as the independent so-called spiracular cartilage of the Batoidei, as the processus oticus palatoquadrati of *Ceratodus*, or as the posttrigeminus portion of the lateral wall of the trigemino-facialis chamber of the Holostei and Teleostei. The processus ascendens palatoquadrati of *Ceratodus* and the pretrigeminus portion of the lateral wall of the trigemino-facialis chamber of the Holostei and Teleostei are either the strict serial homologues of the anterior articular head of the hyomandibula of the Holostei and Teleostei, and hence interarcual cartilages lying between the mandibular and premandibular arches; or they represent the extrabranchial of the premandibular arch here fused with the pharyngeal element of the mandibular arch.

In vertebrates higher than fishes it is evidently possible that any one of the several types of hyomandibula found in fishes might have been independently developed, or, the line of descent of these vertebrates not being known, have been retained by inheritance, and the varying relations of the nervus facialis to

the columella auris of amphibians, which is generally considered to represent the hyomandibula, indicate that the dipneustean and crossopterygean types of hyomandibula are each represented in these animals, and that there is still another type which corresponds to the posterior articular head, alone, of the teleostean hyomandibula.

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CILIARY MECHANISMS OF LAMELLIBRANCHS

WITH DESCRIPTIONS OF ANATOMY

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SEVENTY-TWO FIGURES

INTRODUCTION

In 1900 the writer published a brief account of a complete ciliary mechanism for the removal of waste material from the bodies of lamellibranchs. This involved all ciliated parts of the mantle and visceral mass, certain tracts of the palps, and in one case, the gills. I believe this to have been the first published statement of the existence of such a mechanism, the fact of its presence in lamellibranchs not previously having been known.

Nearly two years ('01) later M. Stenta published a preliminary paper on backwardly directed currents seen in Pinna and some other forms and in 1902 a more detailed paper on the same subject in which a short review was given of the very meager literature relating to the ciliation of organs in the mantle chamber (except the gills, on which more has been written). M. Stenta describes in these papers not merely the mantle ciliation, but also mentions features of the ciliation of the gills, in several forms, and makes some reference to the functions of the palps, without, however, going into the matter fully. A complete account of the operation of ciliary mechanisms for the collection and ingestion of food, or of the disposal of objectionable matter, was not attempted.

In 1903 the writer gave an account of the removal of waste material from the mantle chamber of *Venus mercenaria*.

In 1910 I published a statement showing the palps to be the organs which determined whether gill collections should be carried to the mouth or sent to outgoing tracts, and describing

the function of food collection, and of the disposal of undesirable material, in some detail ("Shellfish industries"). The volume of material was there shown to be the determining factor in the disposal of collections.

Mr. J. H. Orton, in 1912, published observations on the feeding of lamellibranchs, giving particular attention to the function of the gills, in several forms, but noticing, also, some features of mantle ciliation.

So far as I am aware, these accounts are the only ones which refer to a ciliary mechanism for the removal of foreign matter from the mantle chamber.

The mechanism for the removal of objectionable material, brought into the mantle chamber of bivalves by the incurrent stream, was first seen by the writer, in *Yoldia*, in 1898. Since that time, all the coasts of the United States have been visited, as many as possible of their lamellibranchs being studied, until a very large amount of material has been collected, much of which should have been published earlier. Observations on the collection and ingestion of food have been made at the same time, and the present paper gives a brief account both of the collection of floating material, and of its disposal, in the more interesting of the forms in which the mechanisms have been studied. It is also the aim here to illustrate in the figures, features of the gross anatomy of several forms, especially from the Pacific, which have not previously been described. All observations are recorded as fully as possible in the figures, and they are depended on to give much of the information collected on anatomy and ciliation, which is not recited in the text.

METHODS

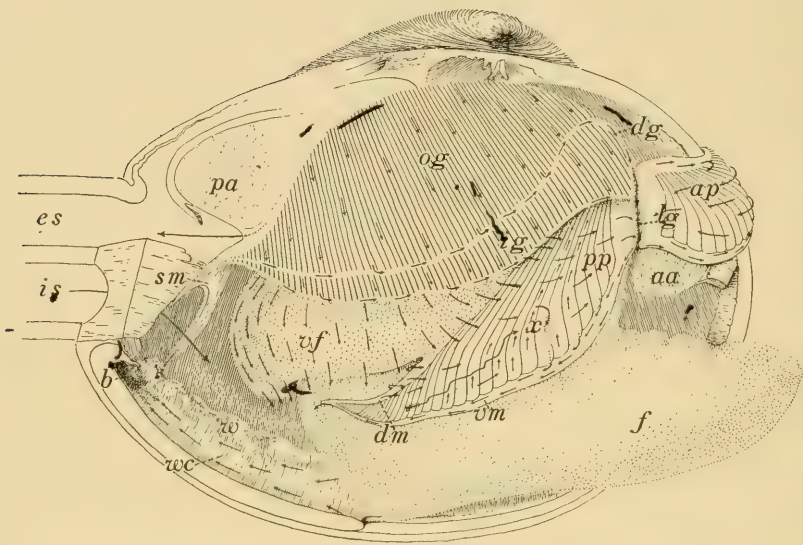
Powdered carmine has universally been used to demonstrate currents caused by ciliary action. It was often useful here, but its grains are very small, and it is so light that small quantities are directed to a desired point with difficulty. It also floats in water *currents* produced by cilia, and then does not always follow the course taken by objects actually resting on the ciliated sur-

face itself, hence errors may easily arise from this. A very fine black sand obtained in Puget Sound and elsewhere, was more often successful, sometimes revealing the presence of ciliation that carmine would not show at all. In demonstrating the fact that there is no selection or separation of food from other water-borne substances, but that the volume of material alone determines the courses that it will take, masses of diatoms gathered by a silk tow-net, were used.

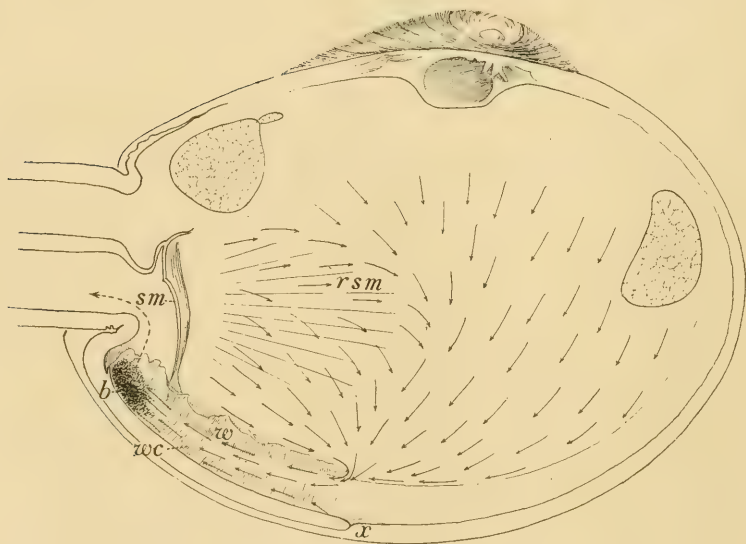
Broad currents were followed by means of hand lenses; minute streams, such as those in the grooves between palp folds, often being demonstrated with a compound microscope. Some of these latter, however, were clear enough to be seen by the unaided eye. Tissues, especially palps, were sometimes removed and pinned out in a dish for observation. Most of these studies were made without the aid of the binocular dissecting microscopes which have recently appeared, and have proved to be ideal aids in work of this nature.

Difficulties. The apparent ease of observing currents may lead to carelessness. Water-borne particles often appear to be cilia-borne. There is a great amount of variation in the intensity of action on the same region in different individuals. The greatest care of the living material having been observed, even the mantle, in certain specimens, will show little or no movement, while it will be perfectly clear in others. The palps present many difficulties. In *Unio*, for example, material ultimately reaching the mouth from the distal oral groove, must pass through a notch to reach the lateral groove. Usually it will be seized here by outgoing currents on the palp margins and carried to the palp tips, and it is only now and then that it may be followed onward in the groove.

Most of the currents to be described have been determined by scores of observations, but of necessity, a few have been followed much less often. Possibly the errors are mostly those of omission. Dorsally and ventrally directed currents on palp grooves, for example, were clearly understood only after most of the forms had been examined, and it has not been possible to re-examine them all with a view of finding these currents.



1



2

A definition. In most forms, the narrow path traversed by material on its way to the mouth is very long. For convenience, it has been necessary to distinguish three regions in this tract, which may be called the oral groove. The first, always very narrow, is that nearest the mouth. It lies between the bases of the palps, which are here without folds. This may be called the proximal oral groove (*pg*, fig. 23). The second is the extension of the groove upward on the side of the body, where palps are widened and united—the lateral oral groove (*lg*, fig. 1). The third is a groove in the mantle wall, close to, and parallel with, the anterior edge of the inner demibranch, found in forms in which the outer demibranch does not extend so far forward as the inner—the distal oral groove (*dg*, fig. 1). In some forms (*Mytilimeria*), the lateral portion of the groove is absent; in others (*Mytilus*) there is no distal portion, while in the majority, all three regions are found.

I am greatly indebted to Dr. W. H. Dall for the identification of several of the forms studied, and to Dr. Fred Baker, of San Diego, whose hospitable door is always open to visiting zoologists, and who identified my San Diego Bay forms, and made it possible for me to study them.

GENERAL ACCOUNT OF CILIATION OF THE BODY OF LAMELLIBRANCHS

Schizotherus nuttallii Conrad, var. *capax* Gould

The location and function of cilia tracts on the outer surfaces of the bodies of lamellibranchs, are much the same in all species. They perform two primary functions, namely, the carrying of

Fig. 1 *Schizotherus nuttallii*, var. *capax*; view of the right side, valve and mantle being removed; *aa*, anterior adductor; *ap*, anterior palp; *b*, bay of mantle; *dg*, distal oral groove; *dm*, dorsal margin of palp; *es*, excurrent tube of siphon; *f*, foot; *ig*, inner demibranch of gill; *is*, incurrent tube of siphon; *lg*, lateral oral groove; *og*, outer demibranch of gill; *pa*, posterior adductor; *pp*, posterior palp; *sm*, siphon membrane; *vf*, vascular fold; *vm*, ventral margin of palp; *w*, wall of waste canal; *wc*, waste canal; *x*, point on palp face where folds are parted.

Fig. 2 *Schizotherus nuttallii*; ciliation of left mantle fold; *rsm*, retractor muscle of siphon; *x*, posterior end of foot opening of mantle, its folds being fused behind this point; other letters as in figure 1.

food to the mouth, and the removal of material when it has become too abundant. Nowhere, perhaps, are cilia tracts to be seen more clearly than in the immense Washington or gaper clam, *Schizotherus*, of Puget Sound. Fullgrown individuals of this species average about two pounds in weight, and certain ones have been found that considerably exceeded this weight. *Schizotherus* is particularly favorable for a demonstration because the palps, bearing the most important and complicated of the ciliary mechanisms, are relatively and actually of immense size.

Visceral fold. The creature is peculiar in the possession of an immense vascular fold which arises from the visceral mass (*vf*, fig. 1), a structure not previously described, so far as I know. Its base, or line of origin, is very broad, and extends from a point far forward on the side of the body, around posteriorly to a similar point on the other side. As shown in figure 1, it is connected for a great distance with the dorsal margin (*dm*) of the posterior or inner palp (*pp*). That it is a posterior extension of the palps themselves, cannot be determined without embryological evidence. In some species, as *Zirfaea* (fig. 55), *Pholadidea* (fig. 53), *Barnea* (fig. 59), and others, there is a more or less extensive attachment between the upper margin of the palp and the visceral mass, and it is conceivable that membranes formed by such an attachment might be extended entirely around the visceral mass, giving rise to an organ like this found in *Schizotherus*. But there is no justification for an opinion in the comparison, and it may be that this curious organ has had an independent origin, and has been secondarily united with the palps.

As to function, the organ, as shown in figure 1, has developed cilia tracts used in collecting material which, anteriorly, is passed on to the palp surfaces, ultimately, if very small in amount, reaching the mouth. Posteriorly, collections are discharged from its free margin into the water filling the mantle chamber, and are subsequently removed from the body. Its inner surface seems not to be ciliated. The organ is thin walled and contains a very large quantity of blood, and being immersed in water, this must become oxygenated. But it seems very doubtful if ciliary or

respiratory functions performed by the organ are physiologically important, for *Schizotherus* possesses, well developed, all the other organs used by lamellibranchs for these purposes. If the vascular fold has other functions, however, that will better account for its presence and its great size, they have not been observed.

Freedom of organs in the mantle chamber

On removing a valve of the shell, it is very noticeable that the ciliated organs lie against each other—the mantle against the outer demibranch, outer palp, and sometimes the visceral mass; the outer demibranch overlies the inner, and the latter rests on the visceral mass. The question arises: How is interference with ciliary function prevented when ciliated surfaces apparently are applied to each other?

It can be seen in several forms—*Pecten*, for example—that in a natural position, with valves more or less separated, the mantle chamber is spacious enough to allow all the organs to extend themselves without touching each other. When it is desirable, such a position is maintained through muscular action, for all gills and palps are capable of complicated and extensive adaptive movements. Frequently, when it is of advantage, as when an excessive load of material is placed on the gills, these organs move so as to touch visceral mass or mantle, and their collections are transferred to the surfaces with which contact is made. Palps frequently are drawn away from gills so as to avoid receiving material from them, and specific instances of these muscular aids to the ciliary mechanism will be mentioned.

The mechanism for food collection

Gills. It has long been known that the floating diatoms which constitute almost the entire food supply of lamellibranchs, are collected by the gills, passed by ciliary action from them to the inner surfaces of the palps, and between these lip-like organs to the mouth. The progress of carmine, or other material used

experimentally, may easily be traced on the gills, but the very few observers who have examined the palps, have been confused by the numerous currents found there, and we have but very meager accounts of what has been supposed to occur on their surfaces. In *Schizotherus*, as in other lamellibranchs, certain cilia on the gill filaments drive the water of the mantle chamber into the basket-like interiors of the demibranchs, thus drawing a current through the incurrent siphon tube, and forcing one out through the excurrent, which is reached by the epibranchial chamber at the base of each demibranch. Suspended particles in the water, striking the mucus-covered gill surfaces, adhere to them, and, if in relatively large quantity, cause an instant local outpouring of a large supply of mucus. Experimentally, relatively enormous quantities of this secretion may be obtained from them in a short period. Particles, entangled in mucus, are carried to the free margins of all faces of both inner and outer demibranchs, then forward in shallow open grooves. The anterior palp (*ap*) in the figure, is folded forward. In its natural position, it lies posteriorly, and over the posterior palp, the edge of the inner demibranch lying between them so that material from its groove is easily transferred to their applied faces. The edge of the outer demibranch (*og*), however, lies entirely above the palps, and particles in its grooves are carried upward, around the front of the inner demibranch, in the distal oral groove, and then into the lateral oral groove, made by the union of the palps, on the side of the visceral mass. In many species, the outer demibranch has this position, as figures of several forms in this account show. Material transferred from gill to palp (near reference letters *ig*), as well as that coming from the vascular fold, starts across the large folds of the palp toward the lateral oral groove (*lg*). Whether it will continue in this course depends on conditions that, experimentally, may be determined at will. Courses taken by particles are the same on both palps. For convenience, the posterior is most fully shown in the drawings.

Palps. These organs in *Schizotherus* are of great size, and are capable of considerable contraction and extension. On the apposed faces are large folds, separated by deep grooves, while

the outer faces are smooth. Each fold is laid forward so as to cover the groove lying anterior to it, as well as a part of the next fold (fig. 3). The folds are not straight, but bend anteriorly as they are traced from their ventral ends, where they are much larger, toward their narrow dorsal ends. It will be observed that these folds do not cover the entire face of the palp. On its margins, above and below the folds, are narrow, plain tracts (*dm* and *vm*), and the folds cease, also, a little distance from the lateral oral groove. The course taken by food on the palps, whether received from the inner gill, or, in *Schizotherus*, from the vascular fold, is directly forward across the folds, as indicated by a set of arrows in figure 1. It is carried into the lateral oral groove, then downward, and in toward the median plane of the body, along the proximal part of the groove, to the mouth. Food collected by the outer demibranch, coming down the distal groove to the lateral, also takes the same course. In their passage across the palp folds, particles are influenced by narrow lines of cilia found along the centers of the folds, and indicated by small, upwardly directed arrows. These lines are very narrow, and particles carried anteriorly across the folds by the general sweep of ciliary action, are usually affected for a brief period by these cross tracts. The course taken by a small particle placed on the palp surface is indicated by the large feathered arrow. It will sometimes pass over several folds without feeling the upward thrust of the fine cilia lines, and then may be carried for some distance on some one line. The utility of these narrow cilia tracts in the centers of folds, is without doubt, in keeping food material away from the ventral margin (*vm*); for should it touch this, it would at once be turned backward and thrown off the posterior end of the palp on to the mantle, which, in turn, would cast it from the body. Yet the palp folds of very few lamellibranchs have been observed to possess these upwardly directed tracts. They were first seen after many forms had been studied, and in a good many cases, a re-examination of palp folds was not possible; but they probably are absent from most palps, for folds of these organs are usually very narrow, and material is carried to the lateral groove with certainty.

The progress of material along the proximal part of the oral groove and its disappearance into the mouth, is not easy to follow, for reasons that will appear; but it has been seen clearly in *Schizotherus*, *Mytilus*, *Mytilimeria*, *Pecten*, and other forms.

The removal of material not used for food

An observation of waters in which lamellibranchs live, whether of lakes, rivers, or seacoasts, will reveal, through the seasons, many great changes in conditions, such as variations in temperature, salinity, or food supply, and there is still much to be learned of the effects of such changes on these forms. There is one condition, however, the effect of which on lamellibranchs can now be described in detail—the periodical loading of waters with mud or fine sand which occurs everywhere as an effect of rains, tide currents, or wave action. No waters are clear all the time, and most of them, even on rocky seashores, sometimes bear very large quantities of sediment for comparatively long periods.

Without doubt, sediment-laden waters present a serious problem to lamellibranchs, which feed on floating organisms, and if the cilia tracts, above referred to, were the only ones in operation, mud or sand, as well as diatoms, would of necessity be carried into the digestive tract as long as the valves of the shell remained open. I have found that species of the genus *Macoma*, of the Pacific coast, habitually take beach sand into the digestive tract until it is distended from stomach to anus, but I have observed no other cases of the sort. It is true that sand grains in relatively small quantities are usually found in the stomachs of lamellibranchs; but it appears to be perfectly certain that generally the ingestion of quantities of material, other than diatoms, is injurious, and is avoided as much as possible. But to close the shell to prevent the entrance of water for long periods, would interfere with respiration. While some lamellibranchs are able to survive for days, or even weeks, in cold weather, when removed from the water, experience with oysters and clams show that adult individuals, at least, are injured by the treat-

ment. As a matter of fact, except when waters are very heavily laden with sediment, lamellibranchs keep their shells open, and allow large quantities of material to be brought into the mantle chamber. This is deposited on the surfaces of all organs exposed, but especially on the gills, or food collecting organs. Except in the cases of *Yoldia*, *Monia*, and *Pecten*, among the forms examined, gill collections can only be moved on to the palps, though experiment would indicate that exceptionally large quantities might fall off from their margins, the masses being too great to be retained by their grooves.

The surfaces of all organs exposed in the mantle chamber produce mucus which is poured out in response to the touch of foreign particles. This is true of mantle and visceral mass, as well as of gill and palp, the adaptation everywhere being the same—the entanglement of solid substances.

Mantle ciliation. Considering the organs concerned in the removal of material from the body, the mantle folds, one of which is illustrated in figure 2, bound the great cavity, and much of the material brought in by the waters adheres to them. At once, mucus and entangled particles begin to move downward, and toward a point near the center of the ventral mantle edge. Figures 1 and 2 attempt to show that the mantle edges are fused from a point, *x*, posteriorly. At the sides of these fused ventral margins arise erectile walls, the left one, *w*, being shown in the figures. These walls are united at the base of the siphon, slightly above the reference letter *b*. They may be elevated and bent toward each other until they almost or quite meet. (Their actual union has been observed in *Mactra*.) They thus enclose a chamber or canal which will be called the waste canal (*wc*). Similar structures occur in other forms to be described. The collections of the mantle folds enter the open anterior end of the canal, as indicated in figure 2, and are carried swiftly backward to be accumulated in a mass at *b*. This mass may become nearly half an inch in diameter in *Schizotherus*.

In figure 2, and other figures showing the direction of mantle currents, it will be noticed that immediately in front of the base of the incurrent siphon through which the stream enters, the

arrows point forward, as well as downward. The reason for such a roundabout course seems to be that particles could not well be carried directly downward across the current; so they proceed with it for a distance before turning posteriorly.

Siphon membrane and waste canal. A waste canal occurs in several species. Wherever it is present there is also a well developed curtain-like structure (*sm*), at the base of the incurrent or branchial siphon. This siphon membrane may be raised to admit the incurrent stream freely, or may be drawn downward so as to throw the stream toward the mantle edges. It seems probable that its function is to throw the current downward on to the mantle edges, and away from the gills, when much sediment is present. A relatively large amount of it would then be deposited on the mantle, and would quickly be taken backward to the small bay *b*. But a downwardly directed current from the incurrent siphon would tend to wash the mantle collections forward in the mantle chamber. In order to prevent this, the covering walls of the waste canal have been developed. The siphon membrane probably should be regarded as an organ developed to aid in the removal of waste matter.

An examination of figure 38 will show an exception to the rule that siphon membrane and waste canal occur together; but this cannot be used as an objection to the explanation offered of the function of these organs. *Cardium* buries only a small portion of the shell in the bottom. The posterior end of the body lies in the water, and mantle collections, instead of being held to accumulate in a mass, to be discharged through the incurrent siphon, as is usual in lamellibranchs, are steadily carried over the mantle edges to the exterior, on a ciliated tract lying below the siphon membrane, where the waste canal is usually found.

All the mantle collections finally arrive at the base of the incurrent siphon, where they are out of the way of the incoming stream. In burrowed forms, the mass cannot be discharged between the ventral shell edges as in *Cardium*, *Unio*, and others, because of the close investment of sand, or fused mantle edges. The walls of the siphon itself are not ciliated, for it would be impossible for cilia to move material against the stream in the

tube. When a sufficient quantity has accumulated, the adductor muscles suddenly contract and pressure enough is developed on the water in the mantle chamber to reverse the stream in the incurrent siphon and throw a jet from it with great force. Being in a favorable position, the mantle accumulation is caught up and discharged through the siphon tube.

Except a narrow border on its free edges, the entire inner surface of the mantle is ciliated, the only function of its cilia being to collect matter for removal. Attention is again called to the fact that, for some reason, there are times when, even on a large ciliated surface, it is very difficult to demonstrate the cilia currents. Trouble was experienced in making out the mantle ciliation of *Schizotherus* at Orcas Island, with light material like carmine, while later it was successfully employed at Eagle Island; and yet it is not possible that locality had anything to do with the matter. Again, when carmine remained stationary, or moved feebly, the relatively enormous weight of a whole teaspoonful of sand, thrown on to the mantle, was moved without difficulty. Everywhere, too, individual differences in the rapidity of movement are found, though whenever currents can be demonstrated at all, they are apparently always the same in like regions.

Outgoing tracts on the palps. Cilia tracts are much more complex on the palps than on other organs, not excepting the gills. When a quantity of carmine settles on the faces bearing the folds, the rapid twists and turns of particles presents a spectacle of the utmost confusion, and it has required a large amount of observation to demonstrate positively the hidden uses of individual currents. While cilia on other organs move material only toward or away from the mouth, the palp cilia perform both these functions, and the mechanism is so complicated that nervous and muscular aid is necessary. It seems certain that the great lateral extension of the palps, and indeed their entire organization, has come about as the result of a demand for more and more perfect ciliary activities. They have become the controlling organs in feeding and in the removal of objectionable matter from the mantle chamber. Except in a very few cases, their action alone

determines whether food shall be taken, or all material rejected and sent outward.

The operation of palp tracts for carrying food to the mouth has been described. Besides these, there are two other tracts, the function of which is to remove objectionable material from the palp surface. The most conspicuous of these is the ventral margin (*vm*), the entire surface of which is covered with cilia which lash posteriorly and carry material out to the narrow, free end of the organ, where it is cast off into the mantle chamber, and disposed of as previously described. Streams on this ventral margin are very rapid and powerful in all lamellibranchs. In one case among those examined (Chama, figs. 39, 40, 41) the direction of the stream is the reverse of its usual course, though its function is the same. A set of outgoing tracts is also found deep in all grooves between the palp folds of *Schizotherus*; and on them material is, at times, conducted from the palp surface to the ventral margin, of which these tracts are tributary streams. Here, then, on the palp face, are four distinct cilia systems. One is a general ciliation of the exposed faces of all folds, directing material anteriorly toward the mouth. A second consists of narrow, independent streams, one in a slight depression on the middle of each fold, all crossing the first system, and directed dorsalward and forward. A third is a single broad band on the ventral margin, with the stream directed posteriorly. The fourth system is comprised of rather broad and powerful streams, joining the ventral margin, and ordinarily lying hidden in the deep grooves between the folds. They conduct material ventralward. When a pinch of sand is thrown on an exposed palp, and all these systems are observed in operation at once, the scene of confusion may be imagined.

The adaptation in the case of each system, however, is clear. They operate under different conditions. It is my belief, after a good many years of observation, that lamellibranchs are able to feed only when the surrounding water is relatively free from solid particles—just how free, in a given case, I am not able to say, and the difficulties in determining the matter are great, if not insurmountable. What actually occurs on the palps, under

varying conditions is this: When waters are relatively clear, small objects, such as diatoms and minute sand grains, or, indeed, any small, chemically non-irritating particles of any nature, are brought to the palp folds from the inner demibranch a few at a time, and cross them toward the mouth. On the way, they are moved dorsalward a little at a time, by the narrow, exposed tracts which cross the first, and thus are kept away from the ventral margin. On reaching the lateral groove, they are joined by the scattered particles collected by the outer demibranch, and, deep in the bottom of this groove, they proceed to the mouth. Even here, close to the mouth, if their mass extends out of the bottom of the groove, they touch outgoing tracts, as will be illustrated in the case of *Mytilus* and other forms, and are pulled out and sent away. Everywhere on the palp, a safe journey to the mouth depends on the small volume of the moving mass. I have witnessed the entire journey from gills to digestive tract many times in several species, and the success of the experiment always depended on the presence of a very small amount of material.

Suppose now that conditions are changed—that the water becomes filled with silt particles, or, in an experiment, with carmine grains or with a mass of diatoms. The outer demibranch makes its collection as before, and, if this is not too heavy to remain in the groove on its margin, passes it to the palp folds, of course entangled in mucus. It starts across these, uninfluenced by the upwardly directed tracts, on account of its mass and extent. But it proceeds only a short distance before muscular movements are observed in the palp. These are two in number in *Schizothorus*, and their adaptations are beautiful in their efficiency. Somewhere under, or in front of the advancing load, the anterior edges of one or more folds are drawn backward and upward, so as to expose the ventrally directed tracts which have been concealed in the grooves (as at *x* in figs. 1 and 3). Parts of the common mass of mucus and entangled particles are driven with great certainty into the grooves, and the whole is then dragged swiftly to the ciliation of the ventral margin, there to be carried backward, and thrown into the mantle chamber.

The second muscular movement clears the palp surface even more swiftly, and is usually put in operation when an exceptionally large amount of material appears on the folds. It consists in a rolling upward of the ventral margin—or a part of it—until the mass on the folds is touched and lifted off. More often, in other forms than *Schizotherus*, in which the lateral part of the palp is continuous with the vascular fold, the palp is contracted into a spiral, folded face outward, so that parts of the ventral margin touch all surfaces of the palp; and, while thus twisted, the whole is rapidly cleaned of every particle adhering to it. Everything quickly reaches the tip, and is thrown off.

Large collections of the outer demibranch are usually taken directly from the distal groove which runs down anterior to the inner, by cilia of the mantle anteriorly, as will be described in other forms. If, however, more than an extremely small amount of material is placed in the lateral groove between the palps, some of it touches the ventral margin, and all is carried away.

Oral objection has been made to my contention that oysters are able to feed only when waters that they inhabit are comparatively clear. This is based on the fact that waters on certain oyster fields are sometimes muddy for long periods, and also that in waters frequently muddied, there are found, at times, in the stomach contents so many more sand grains than diatoms, that the latter cannot be counted. The conclusion therefore is that oysters feed normally in muddy waters.

It may be answered that no one has ever determined in the periods during which oysters are known to have grown—the summer months in the North—the duration of the times in which waters have been turbid or comparatively clear, nor at any time, the amount of turbidity. Changes in the amount of suspended matter in all waters, salt or fresh, are frequent, and especially along seashores everywhere, for here wave and tide action make themselves felt. Very marked changes in the degree of clarity may be observed almost daily, even in the small, sandy-bottomed lakes of Florida, in which, if anywhere, one might expect constancy in this respect. The subject has not been studied carefully. From my own general observations, I believe that the

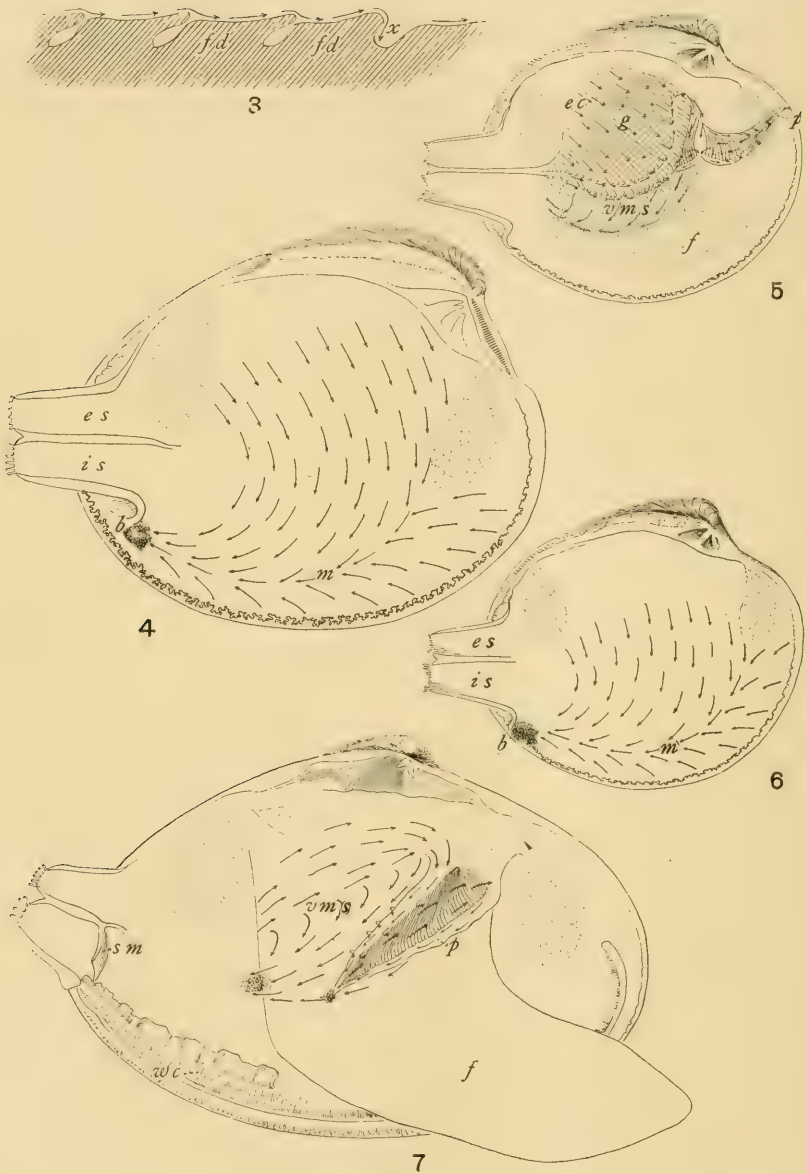
muddy waters of the Chesapeake are, in any given locality, comparatively clear much of the time. It may be doubted if the Chesapeake ever becomes as muddy as the waters over the oyster fields near the mouth of the Mississippi; and yet even here, a large part of the time, they would be spoken of as clear.

Secondly, in the contention that stomach contents sometimes contain a larger volume of sand than of diatoms, there appears no argument against the view that lamellibranchs feed only in comparatively clear water, though, of course, they will receive some nourishment if there are any diatoms at all in the stomach. It will appear from the foregoing account of the palp functions that the volume of the material, and not its nature as possible food, determines whether or not it shall be taken into the stomach. If there is sand in the stomach of a lamellibranch, it has been taken in a very little at a time, and I know of no means by which this would be possible if very much at a time were received into the mantle chamber. Apparently, to adopt the view that feeding is possible in very muddy waters, is to make the seeming function of the outgoing cilia tracts quite meaningless. And yet there remains the anomalous, not to say disconcerting case of the genus *Macoma*, of the Pacific, all the species of which I have found to possess outgoing tracts, and to be habitual sand eaters. It is difficult for me to believe that there is not some feature of the ciliation of these forms that has escaped my attention, which, if known, would explain this peculiar habit. These forms will be described subsequently.

Visceral mass A ciliation of the visceral mass could not be demonstrated in the specimens of *Schizotherus* examined, though possibly it is present, as in the majority of bivalves. The surface of the foot in all lamellibranchs seems to have lost its embryonic ciliation.

Venus mercenaria

Gills. Material on outer and inner lamellae of both demi-branches is moved to the free edges, and forward to the palps, as in *Schizotherus*. A tract between the gills, at their bases, moves



particles forward to meet those brought on the margin of the outer demibranch. This is all moved downward to the lateral groove. Apparently there are no similar lines at the base of the outer lamella of the outer demibranch, or that of the inner lamella of the inner demibranch.

Palps. On the outer faces of the palps, cilia streams move around the dorsal margins to the inner, or apposed faces. Most of the material usually moves on to the palp folds, and is then disposed of as in *Schizotherus*. Whether, as in that form, there are ventrally directed streams between the folds, and dorsally directed ones on their crests, was not determined. The palps are narrow, and respond to touch with so great contortion, that they are not easily studied.

Visceral mass. The ciliation of the visceral mass is much like that of *Mactra*, though movement of particles is more directly toward its posterior wall, from which it is thrown off to the mantle. Here, also as in *Mactra*, some part of the collection may be sent to the dorsal margin of the palp, and if not of too great volume, may ultimately reach the mouth.

Mantle. In *Venus* and several related forms, as well as in some other groups, a distinct line, or narrow path (*m* in fig. 4), extends over the mantle from a point near the lower edge of the anterior adductor to the base of the incurrent siphon (*b*). There is a general ciliation, without definite lines or tracts, covering the remainder of the mantle wall, and, as indicated in the figure, everything on them is moved to the line. An appearance of definite lines or tracts is often seen on a surface possessing a general ciliation, because a mucus mass may be drawn out into a long thread. Waste accumulates at the bay *b*, and on contrac-

Fig. 3 *Schizotherus nuttallii*; section across palp folds; *fd*, folds; *x*, point where folds are parted to expose deep cilia tract;

Fig. 4 *Venus mercenaria*; mantle ciliation; *b*, bay of mantle; *es*, excurrent tube of siphon; *is*, incurrent tube of siphon; *m*, mantle wall.

Fig. 5 *Chione fluctifraga*; *ec*, epibranchial chamber; *f*, foot; *g*, gill; *p*, palp; *vms*, visceral mass.

Fig. 6 *Chione fluctifraga*; mantle ciliation; letters as in figure 4.

Fig. 7 *Mactra solidissima*; *f*, foot; *p*, palp; *sm*, siphon membrane, *vms*, visceral mass; *wc*, waste canal.

tion of the adductor muscles, is discharged through the incurrent siphon. It does not appear to be discharged between the mantle folds below the siphon, as in *Mactra* and other forms.

Chione fluctifraga

This form was studied at San Diego Bay, California.

Gills. Cilia streams are to the margin on all lamellae. There is a forwardly directed tract between demibranchs, at their bases, but apparently none at the bases of outer lamella of the outer, and inner lamella of the inner demibranchs. The inner demibranch is attached anteriorly to the inner face of the posterior palp, and very close to the lateral oral groove.

Palps. The palp ciliation is like that of *Schizotherus*, except that no dorsally directed currents were seen on the faces of the folds (fig. 5). Though the folds are minute, currents directed downward to the ventral margin in the grooves between them, were clearly demonstrated by the use of heavy, powdered sand.

Visceral mass. The ciliation here (fig. 5, *vms*) is downward and backward, collections being cast from the posterior wall as in *Venus*.

Mantle. Figure 6 shows a ciliation like that of *Venus*.

Chione succincta

The ciliation of all organs is like that of *C. fluctifraga*.

Tivela crassatelloides Conrad

The *Tivelas* are found in California from Santa Cruz southward. They are very abundant at Pismo Beach, and in the market are known as the Pismo clams. The shell valves are extremely heavy. The ciliation of all organs seems to be precisely like that of *Venus*. There is a small siphon membrane, but no waste canal on the mantle. The usual line through the general ciliation of the mantle, found in forms related to *Venus*, is well defined. The tentacles of the mantle margins are tree-like, bearing secondary and tertiary branches.

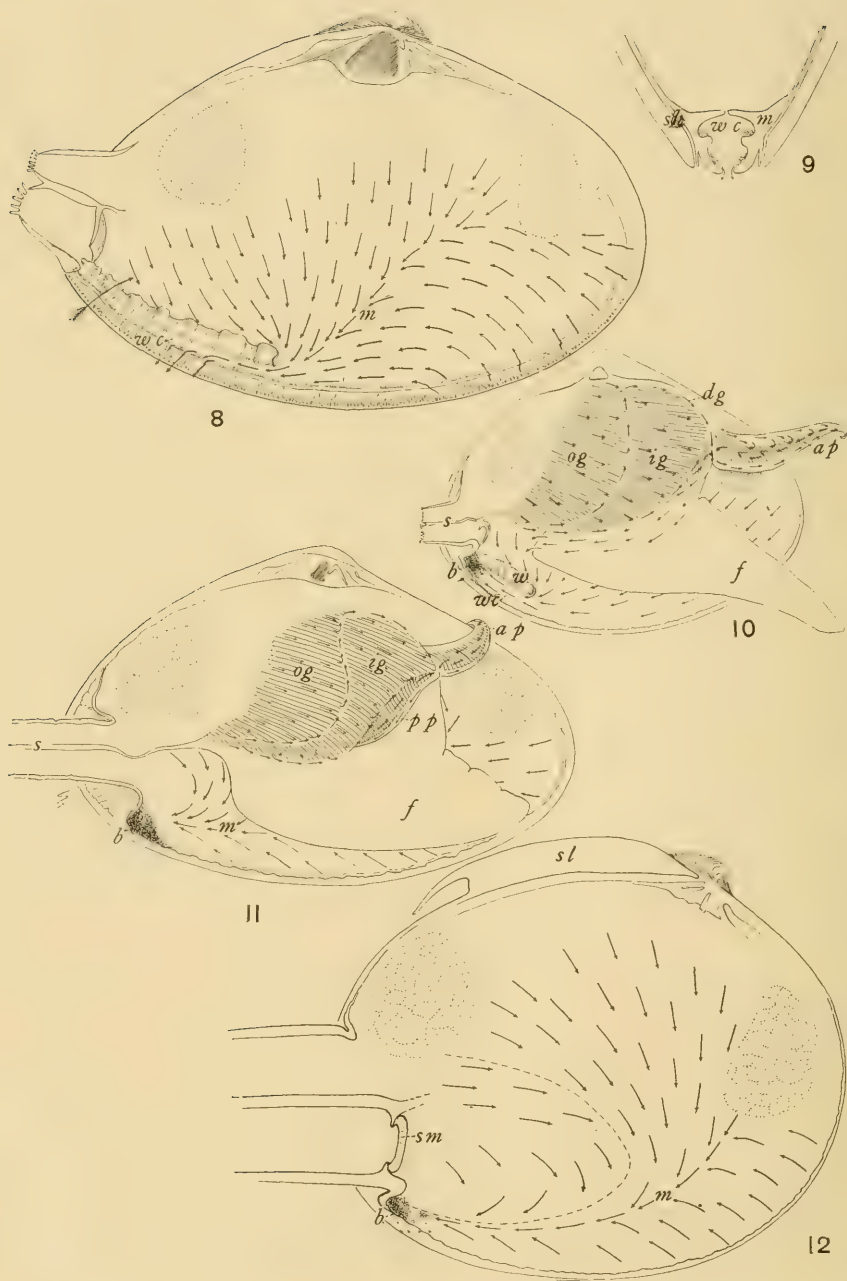
Mactra solidissima

Gills. As in *Venus*, currents are downward to the free edges of the demibranchs of all lamellae, and forward on the margins to the palps. There is also a forwardly directed current between demibranchs at their bases, but none at the bases of the outer lamella of the outer, or inner lamella of the inner demibranchs.

Palps. These organs are long and narrow, and writhe and twist at the touch of small quantities of material. Dorsal and ventral margins are relatively wide. In figure 7, the gills are removed, and the posterior palp of the right side is shown. The course toward the mouth across the folds is indicated, as is the fact that the ventral margin carries particles posteriorly to the palp tip, where they are thrown off into the mantle chamber. The figure indicates, also, that material collected on the back face of the palp—that which is applied to the visceral mass—is carried over on to the dorsal margin of the inner face. Here its general course is backward to the tip, as on the ventral margin. Material carried close to the folds is usually caught up and sent forward across them. On the folded area, as in *Schizotherus*, there is one set of currents directed ventrally, and another dorsally. These were seen, but their positions not clearly determined, before the beautifully clear demonstration on the palp of *Schizotherus* was found. Since then, there has been no opportunity for comparison. It appeared that the ventrally directed currents were on the folds, those toward the dorsal margins, in the grooves, a condition the reverse of that in *Schizotherus*, but this may be an error.

Visceral mass. High on the side of the visceral mass, the trend of a general ciliation is forward (fig. 7). Part of the collection here is sent on to the dorsal margin of the palp, which organ is extensively united to the body wall. Below this, the visceral mass collections are carried to its posterior wall, and cast off to fall to the mantle.

Mantle. There is a single line, or tract, running through the general ciliation of the mantle wall (fig. 8, *m*). Forward, its position is farther from the mantle edge than in *Venus*. Pos-



teriorly, it is directed close to the mantle edge, in order to pass outside of a mantle fold, and into a waste canal (*wc*), as in *Schizotherus*. A sectional view across the waste canal is shown in figure 9, *wc*. That the edges of the mantle folds meet, as is here shown, so as to enclose the waste canal, is not a matter of inference, but was observed on a living individual. A siphon membrane, for throwing heavily laden currents downward, is well developed, and without doubt, the waste canal protects undesirable material from being washed back into the mantle chamber. The mantle edges being free in the region of the waste canal, and *Mactra* not being entirely buried in the bottom, the collected waste is not accumulated at the base of the incurrent siphon, but is carried out, partly by a feeble ciliary action, and partly by muscular contortions of the mantle, between its edges.

As *Mactra* buries only the anterior part of the shell in the bottom, the mantle edges, including their folds, are often, if not always, opened at a point indicated by the feathered arrow in figure 8, to form a supplementary incurrent opening, through which water is drawn into the mantle chamber, as proved by suspended carmine. What advantage is gained by this second passage into the chamber of the mantle, is hard to imagine.

Spisula (Hemimactra) polynyma, Stimpson

This form was taken from the Bay of Fundy.

Gills and palps. As shown in figure 10, the ciliation of gills and palps is like that of *Schizotherus*, except that dorsally directed currents were not seen on the palp folds. In their normal positions, the posterior palps are nearly covered by the inner demibranchs, and the outer demibranchs are removed by a

Fig. 8 *Mactra solidissima*; mantle ciliation; *m*, mantle wall; *wc*, waste canal.

Fig. 9 *Mactra solidissima*; section across mantle edges; *sh*, shell.

Fig. 10 *Spisula polynyma*; *ap*, anterior palp; *b*, bay of mantle; *dg*, distal oral groove; *f*, foot; *ig*, inner demibranch of gill; *og*, outer demibranch of gill; *s*, siphon; *w*, wall of waste canal; *wc*, waste canal.

Fig. 11 *Spisula planulata*; letters as in figure 10.

Fig. 12 *Saxidomus gigantius*; mantle ciliation; *b*, bay of mantle; *m*, mantle wall; *sl*, shell ligament; *sm*, siphon membrane.

considerable distance from the palps, the distal oral groove (*dg*) being long in consequence.

Mantle. The mantle ciliation apparently does not, as in *Maetra*, possess the single, posteriorly directed, cilia tract.

There is a short waste canal (*wc*) into the anterior end of which, in the usual manner, mantle collections are led. As in other forms possessing a waste canal, a well developed siphon membrane is present.

Spisula planulata Conrad

This *Spisula* was studied at San Diego Bay, California.

Gills and palps. The ciliation of these organs is like that of the form just mentioned.

Mantle. There is the single line extending backward to the bay at the base of the incurrent siphon (fig. 11, *b*). Waste canal and siphon membrane, however, are absent. There thus seems to be considerable difference in the ventral region of the mantle in these two species of *Spisula*.

Saxidomus gigantius Deshayes

Gills. Streams on all lamellae are to the margins, in this Puget Sound form.

Palps. The attachment of posterior palps to the wall of the visceral mass is not extensive. The ciliation of both faces is practically the same as in *Schizotherus*.

Mantle. The edges are not fused; the siphon tubes are relatively large; the mantle edge possesses a very wide thickening. As in *Venus*, there is a sharply defined curved line in the general ciliation of the mantle wall (fig. 12, *m*), extending from the anterior adductor to the bay below the incurrent siphon (*b*), where mantle collections lodge before being ejected through the siphon tube. At the base of the incurrent siphon, is a siphon membrane (*sm*) of unusual construction, a circular structure with a central aperture, which is opened wide with a circular outline, or nearly closed into a vertical slit. This structure probably cannot throw the entering stream downward, and there

is no mantle waste canal. It may be that the diaphragm controls the amount of water entering the mantle chamber.

Mya arenaria

This form is found on the Atlantic from Virginia north to Labrador, and on the Pacific, where it has been introduced with Atlantic seed oysters, in San Francisco Bay, Puget Sound, and northward.

Gills. As in the majority of lamellibranchs, cilia streams are downward to the margins on all lamellae. There is an anteriorly directed stream between the bases of demibranchs on each side of the body, but none at the bases of the outer lamella of the outer, or inner lamella of the inner demibranchs.

Palps. The general ciliation (one without definite lines or tracts) covering the outer faces of the palps, conducts collected material around the dorsal edges to the inner or apposed faces. On the dorsal margin of this latter surface, the stream is directed posteriorly, and across to the folds, on to which it is lifted, and is then started toward the mouth. The ciliation of the ventral margin is directed posteriorly to the tip, as usual. Posterior to the middle of each fold, but not deep down in the groove between folds, is a ciliation upward to the dorsal margin, and material which happens to be carried to this margin, is thrown back again on to the folds, as in *Schizotherus*: but ventrally directed tracts, deep in the grooves, were not seen, perhaps because when examinations were made, the presence of such tracts in *Schizotherus* and others, was not known.

Visceral mass. There is a general ciliation over the walls of the visceral mass, collections being directed downward nearly to its ventral margin, then posteriorly to a position nearly opposite the base of the incurrent siphon, *s.* Here all material is brought to a vortex (fig. 13, *v*) a considerable part of it often being collected in a rotating ball before it falls, or is washed off by the incoming stream of water, to lodge on the mantle.

Mantle. The mantle edges of *Mya* and its allies are united except for siphon and foot openings. The great expanse of its

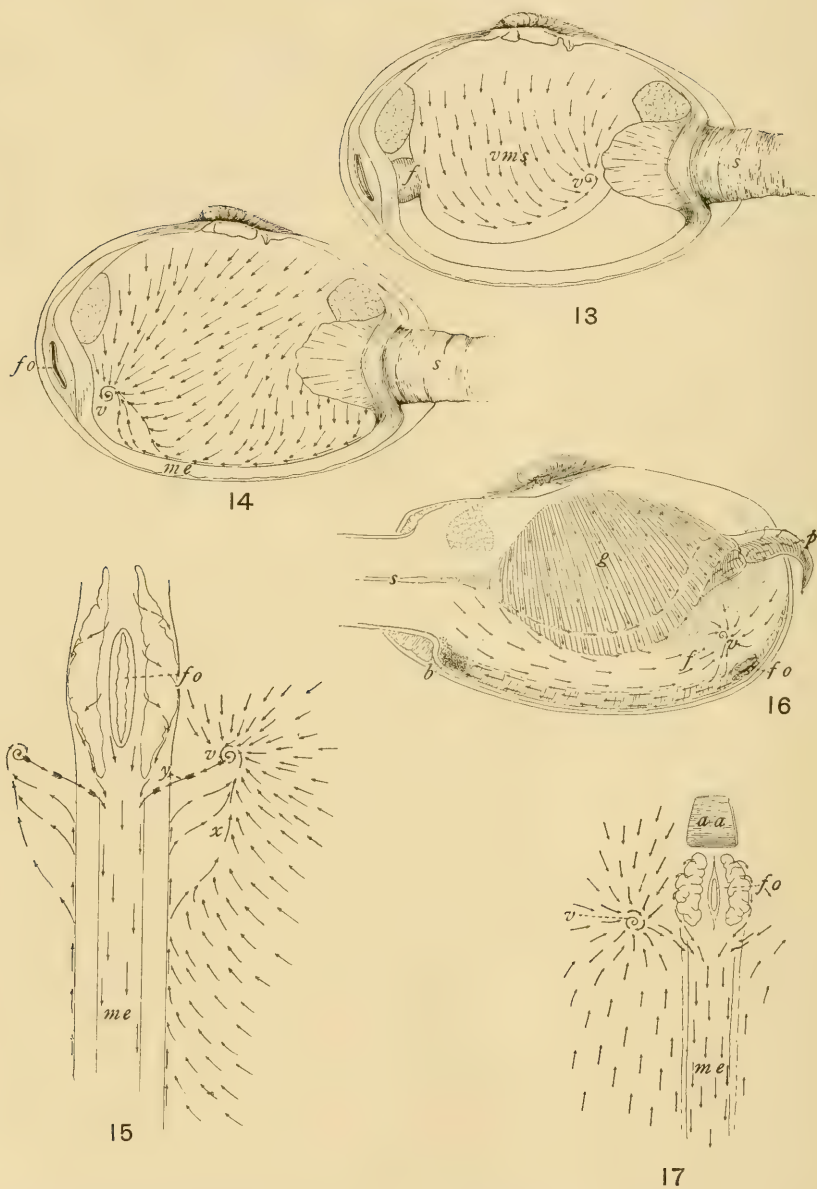


Fig. 13 *Mya arenaria*; ciliation of visceral mass; *f*, foot; *s*, siphon; *v*, vortex; *vms*, visceral mass.

Fig. 14 *Mya arenaria*; ciliation of mantle; *fo*, foot opening in mantle edge; *me*, mantle edge; *s*, siphon; *v*, vortex.

wall possesses a general ciliation (fig. 14), material being carried down, posteriorly, to the thickening of the fused edges, and then sharply forward; while over the upper and anterior part of the wall, everything is carried directly to a vortex (*v*) near the foot opening. Figure 15 represents a ventrally directed view of the anterior portion of the fused edges. In addition to a general ciliation, several distinct lines (*x*) lead to a vortex (*v*), and here accumulate balls of mucus with entangled particles, which sometimes attain a diameter of four or five millimeters. When these whirling balls happen to topple over toward the fused edges, they are caught up by a line of cilia (*y*), and carried on to a broad tract (*me*), which conducts them swiftly backward to a bay below the base of the siphon, as in most bivalves. Ultimately they are discharged through the incurrent siphon as described for *Schizotherus*.

Variation in mantle ciliation of different forms is greater than in other organs. Here the vortices seem to characterize *Mya* and its near relatives.

Platyodon cancellatus Conrad

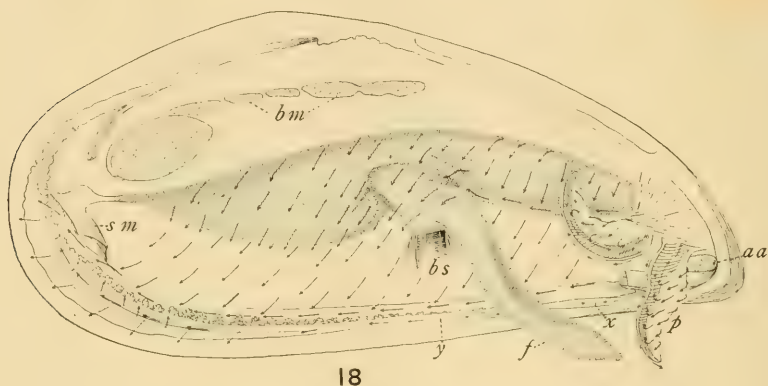
This species occurs on the coast of California. Though found in burrows of soft sandstone, or similar rock—and apparently not elsewhere—it is not a true borer, but a nestler in the excavations of borers. It attains a length of about three inches, and is much like *Mya* in appearance. The ciliation of all of its organs is practically identical with that of *Mya*. The general courses of gill and palp currents are shown in figure 16. On the side of the visceral mass, somewhat below its center, is what appears to be a poorly developed vortex. Very little material stops here, however, most of it being carried down to the ventral wall, a short distance behind the very small, hatchet shaped foot,

Fig. 15 *Mya arenaria*; mantle edge from above; *x*, ciliated tracts to mantle vortex; *v*, single tract from vortex to ventral mantle.

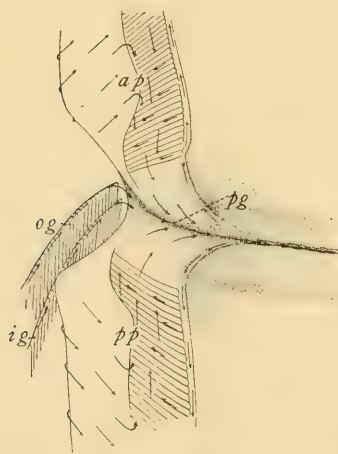
Fig. 16 *Platyodon cancellatus*; *b*, bay of mantle; *f*, foot; *fo*, foot opening; *g*, gill; *p*, anterior palp; *s*, siphon; *v*, mantle vortex.

Fig. 17 *Platyodon cancellatus*; mantle edge from above; letters as in figure 15.

from which position it drops to the mantle. Figure 17 is a view of the region about one of the mantle vortices, seen from above. Except in minor details which a comparison of figures will reveal, the mantle ciliation is like that of *Mya*.



18



19

Fig. 18 *Mytilus edulis*; *aa*, anterior adductor; *bm*, byssus muscles; *bs*, byssus; *f*, foot; *p*, anterior palp; *sm*, siphon membrane; *x*, meeting of currents on mantle edge; *v*, divergence of currents.

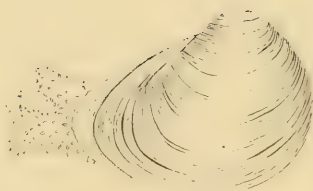
Fig. 19 *Mytilus edulis*; view of palps from below; *ap*, anterior palp; *ig*, inner demibranch of gill; *og*, outer demibranch of gill; *pg*, proximal oral groove; *pp*, posterior palp.

Mytilus edulis and *M. californiana*

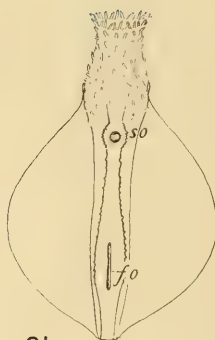
Cilia tracts on most of the organs of *Mytilus* and its allies are clearly defined. Figures 18 and 19 are drawn from *M. edulis*, but *M. californiana* is almost exactly similar in anatomy and ciliation, though it is of immense size, one individual, taken from Puget Sound, having a length of 19 cm., or $7\frac{1}{2}$ inches, and a weight of one pound and nine ounces, others, of several clusters having about the same size.

Gills. The movement by cilia on all lamellae, is to the free margin. The outer lamella of the outer demibranch is not united with the mantle, nor the inner lamella of the inner demibranch with the visceral mass, but at the bases of both of these is an anteriorly directed stream toward the palps. In both species there seems to be no tract between the bases of contiguous demibranchs.

Palps. The apposed faces, bearing the folds, have wide dorsal and narrow ventral margins. The relation of palps to each other and to the anterior ends of the demibranchs (*og* and *ig*) is represented in figure 19, a view of the organs as seen from below. The anterior palp (*ap*) is folded forward. In the center of the figure, opposite the gill, is the groove between palps (the proximal oral groove *pg*) leading to the right toward the mouth. The mouth opening is hidden by the apposition of the palps over it. Material collected on the backs of the palps, is brought over their dorsal edges, and carried diagonally across the dorsal margin to the folds. If very small in amount, it crosses them to the oral groove, and, deep down in this, is carried toward, and into the mouth. Perhaps no part of the ciliary mechanism is so difficult to demonstrate in lamellibranchs in general as this proximal oral groove; but various materials have repeatedly been sent through it, and have been seen to enter the mouth, in all members of this group which have been examined, as well as in several others. The secret of success is the employment of very minute quantities. Not much is required to extend out of the groove far enough to come in contact with, and to be transferred to, outgoing streams, shown here near the



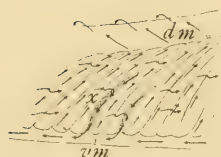
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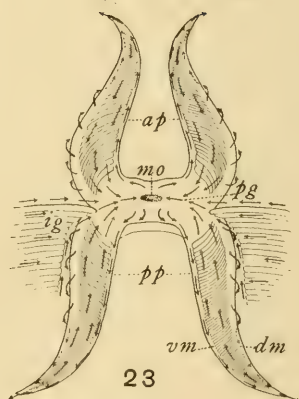
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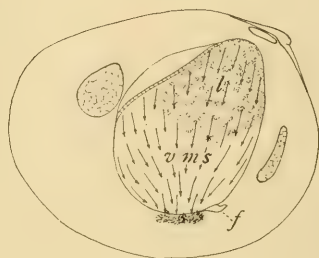
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ventral margins, and more fully in figure 23. With palps in their natural positions, material may be taken from the demibranchs by the palp folds before reaching their anterior ends. When it does reach the ends, however, it is taken off on to the groove between palps, as shown in figure 19.

In each species close to the line where one palp fold overlies another, but apparently not deep in the groove, there is a dorsally directed stream. Streams to the ventral margin, deep in the grooves, have not been observed, but very likely they are present.

Visceral mass. Material collected on its walls is dropped into the mantle chamber from the ventral wall behind the base of the byssus (fig. 18 b), but not anterior to the foot. This last mentioned organ is not ciliated in any full grown bivalve.

Mantle. The mantle edges are free, and there is no sharply defined incurrent siphon opening. There is, however, a large siphon membrane (fig. 18 *sm*). Extensive folds, like those bounding the waste canals of *Schizotherus* and many other forms, are developed parallel to the mantle edge, but there is no waste canal, the mantle collections, instead of being carried ventral to and between them, are carried backward dorsal to their bases and, without halting to collect in masses, leave the mantle chamber directly posterior to the siphon membrane. Because the incurrent siphon is not an enclosed tube, probably these mantle folds tend to shield the waste materials from the broad incoming

Fig. 20 *Mytilimeria nuttallii*.

Fig. 21 *Mytilimeria nuttallii*; ventral view; *fo*, foot opening of mantle; *so*, supplementary mantle opening.

Fig. 22 *Mytilimeria nuttallii*; view of right side; *b*, bay of mantle; *es*, ex-current tube of siphon; *f*, foot; *ig*, inner demibranch of gill; *is*, incurrent tube of siphon; *og*, outer demibranch of gill; *pp*, posterior palp.

Fig. 23 *Mytilimeria nuttallii*; mouth and palps seen from below; *ap*, anterior palps; *dm*, dorsal margin of palp; *ig*, inner demibranch of gill; *mo*, mouth; *pg*, proximal oral groove; *pp*, posterior palps; *vm*, ventral margin of palp.

Fig. 24 *Mytilimeria nuttallii*; detail of palp ciliation; *x*, separation of folds to expose deep cilia tracts; other letters as in figure 23.

Fig. 25 *Mytilimeria nuttallii*; ciliation of visceral mass, *vms*.

Fig. 26 *Mytilimeria nuttallii*; ciliation of mantle edges; *b*, bay of mantle; *g*, posterior ends of gills; *is*, opening of incurrent tube of siphon; *m*, mantle wall; *so*, supplementary mantle opening.

stream of water. It may be reasonable to assume, also, that the siphon membrane functions here, as in *Schizotherus*, in throwing heavily laden water downward. Like many structures, the mantle folds respond locally by muscular contortions whenever they are touched, the fold invariably bending outward to touch the mantle edge, and remaining until the stimulating substance has been forced from between them.

The extreme ventral edge of the mantle has cilia tracts of its own, the adaptation, in the separation of which, at *y*, and their meeting at *x*, is not apparent.

Mytilimeria nuttallii Conrad

This interesting species, specimens of which were taken among the San Juan Islands of Puget Sound, possesses a very thin, white shell, covered by a brown cuticle, and is imbedded in masses of ascidians, from which it gains protection. Shells vary in outline, sometimes being much distorted by pressure of surrounding objects. The typical form is illustrated in figures 20 and 21, the latter being a ventral view, showing fused mantle edges, with a foot opening (*fo*) and a curious small supplementary opening (*so*) near the base of the incurrent siphon. An ossicle, present under the hinge, is fastened to the ligament. The extremely small foot (fig. 22 *f*) has the remains of a groove on its ventral side near the tip, the organ probably having functioned in fastening byssus threads in the young. The anatomy of the form, of which little has been known, is more or less fully shown in the following figures, and perhaps does not need further description.

Gills. The outer demibranch (fig. 22) is narrow and lies dorsal to the inner without covering it. Filaments are not united by ciliated patches as in *Mytilus*, but are united by concrecence. Cilia currents are down to the margin of the demibranch, and are continued downward across the outer lamella of the inner demibranch to its margin. The movement of particles is also downward to the gill margin on the inner lamella of the inner demibranch.

Palps. In figure 22 the posterior palp (*pp*) has been pulled out from its natural position under the inner demibranch, and the anterior palp is folded forward. Figure 23 is a ventral view of the mouth region, with anterior palps (*ap*) folded forward, and shows the relation between palps and the inner demibranch (*ig*). It shows, perhaps better than other figures, the position of the narrow stream running in the proximal oral groove (*pg*) to the mouth (*m*), and the outgoing tracts on both palps above it. In all lamellibranchs the ciliation of the region about the mouth is similar to this.

Material moving forward on the free ventral margin of the inner demibranch may be taken off on to any of the palp folds, to be passed over them to the mouth (fig. 24). Too heavy a load will cause the folds to part and expose ventrally directed streams in the grooves, which function as in *Schizotherus*. But if material is not removed from the inner demibranch margin to the palp folds, it continues forward, and is carried well into the proximal oral groove (fig. 23 *pg*); such a direct communication between gill and oral groove is shown in several of the figures. It is evident that if more than a minute amount of material passes along this very narrow groove, it will be caught up by outgoing currents lying very close to it, near the mouth. This makes it extremely difficult to arrange an experiment so that material may be seen actually to enter the mouth opening.

The ciliation of the dorsal margins of the palps is very unusual in that material is carried over them to the outer faces of the organs, and thence to their tips, the entire outer surface, in each case, directing currents posteriorly.

Visceral mass. Figure 25 shows cilia currents below the line of gill attachment, over the liver mass (in stipple) and sexual mass to the ventral wall immediately behind the foot (*f*).

Mantle. Collections are here moved to the fused and thickened edges, where they are led posteriorly, in the usual manner, to the base of the incurrent siphon (fig. 22). A view of this mantle edge is given in figure 26, in which the very small supplementary opening (*so*) is shown. The significance of this opening and a similar one in *Lyonsia saxicola* was not determined.

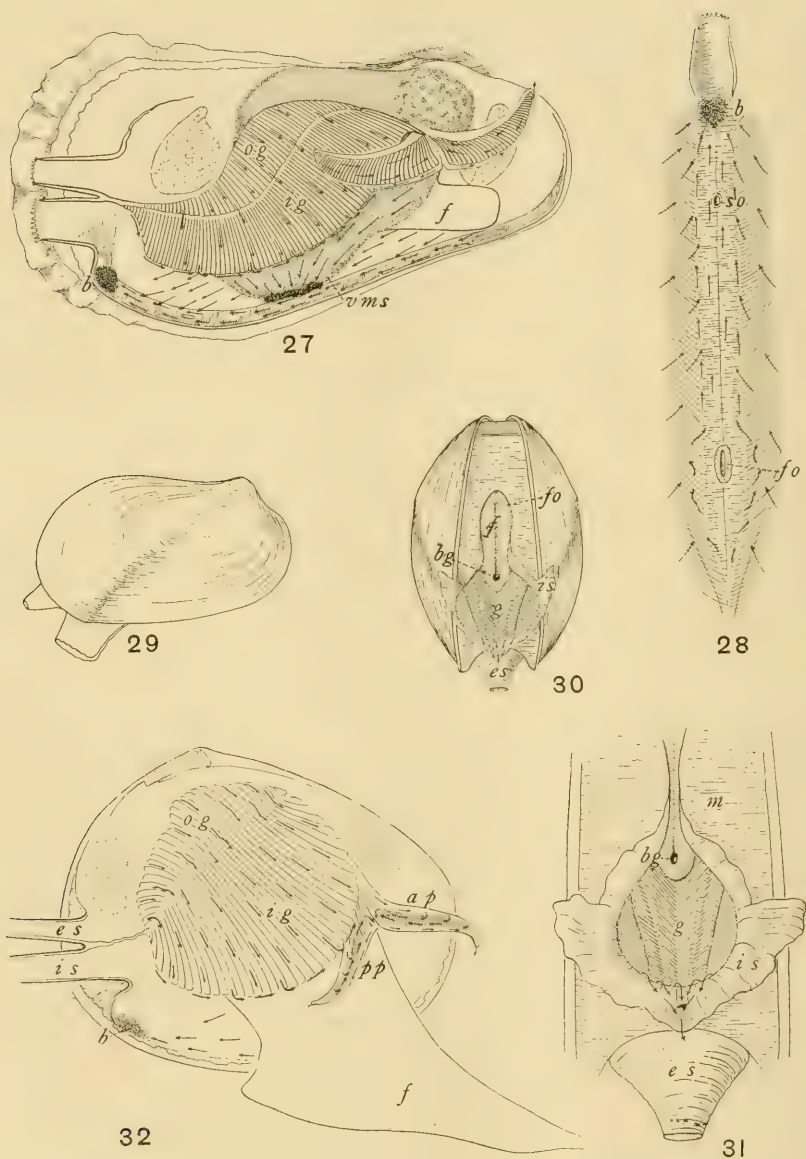


Fig. 27 *Lyonsia saxicola*; *b*, bay of mantle; *f*, foot; *ig*, inner demibranch of gill; *og*, outer demibranch of gill; *vms*, visceral mass.

Fig. 28 *Lyonsia saxicola*; mantle edge from above; *b*, bay of mantle; *fo*, foot opening of mantle; *so*, supplementary opening of mantle.

Lyonsia (Agriodesma) saxicola Baird

This is a North Pacific species, the specimen examined being taken in Puget Sound. There is an ossicle beneath the hinge; a thick epidermis over the shell, extending far beyond the shell ridge posteriorly; and a supplementary opening, posteriorly, through the fused mantle edges, characters found also in *Mytilimeria*. There is a groove for byssus secretion on the under surface of the foot, and the species is hermaphroditic.

Comparing figure 27 with figure 22, and figure 28 with figure 26, it will be seen that the ciliation of all surfaces in *Lyonsia saxicola* is like that of *Mytilimeria*, and one would thus presume that they are closely allied forms.

Lyonsia californica Conrad

This form was difficult to study on account of its small size, the shell being less than an inch in length. The ciliation, however, was clearly essentially like that of the preceding species.

Modiolaria nigra Gray

One specimen was taken from Puget Sound. The shell was covered by a heavy pale green cuticle. This form presents some interesting anatomical features. As shown in figures 29 and 30, the mantle edges are fused anteriorly, but the ventral side of the incurrent siphon is open, and communicates with the foot opening (*fo*). Muscular contraction normally brings the mantle edges together so as to separate these openings; but when water is heavily laden with particles, they form one large opening through which water enters the mantle chamber, as shown in

Fig. 29 *Modiolaria nigra*.

Fig. 30 *Modiolaria nigra*; mantle edge exposed by parting of valves; *bg*, opening of byssus gland; *es*, excurrent tube of siphon; *f*, foot; *fo*, foot opening of mantle; *g*, gills; *is*, walls of incurrent siphon opening; widely opened, the incurrent siphon opening becomes one with the foot opening.

Fig. 31 *Modiolaria nigra*; part of figure 30 enlarged.

Fig. 32 *Semele decisa*; *ap*, anterior palp; *es*, excurrent tube of siphon; *is*, incurrent tube of siphon; other letters as in figure 27.

figures 30 and 31. In the latter case, water enters the foot opening as freely as the incurrent siphon opening, as proved by the use of carmine. The sexual glands fill the mantle as in *Mytilus*. I have presumed ('92) that this shifting in the position of the sexual mass in lamellibranchs is often due to the fact that the byssus muscles come to occupy so much of the space of the visceral mass. *Modiolaria* has a well developed byssus, and a foot with a byssus groove.

The ciliation of the body is much like that of *Mytilus*. Gill currents on all lamellae are to the margins, and forward to the palps. These latter organs are small and hard to examine, but ciliation on them seemed to be as in *Mytilus*. On the mantle walls, material is carried ventrally and then posteriorly. It continues on its backward course, over the dorsal wall of the incurrent siphon (*is*) to the exterior, as shown in figures 30 and 31, against the entering current of water; and unless we except *Mytilus* where the anatomical features of the mantle are not the same, this performance is unique among lamellibranchs examined. It was observed that the effect of the stimulus of a large amount of carmine or sand in the water, was to open wide the connection between the incurrent siphon and foot openings; and that while water entered the mantle chamber freely, a steady stream of waste matter poured out over the dorsal wall of the incurrent siphon.

Semele decisa Conrad

One small specimen from San Diego Bay was examined. The courses of the chief cilia currents are shown in figure 32.

Macoma secta Conrad

This genus is one of the most interesting of those examined on account of its unique habit of taking large quantities of sand into the digestive tract. Some of its five or six species are found from Alaska to Mexico on the Pacific coast. *Macoma secta* (fig. 33), the largest, has a shell 7 or 8 cm. in length. Members of the genus burrow several inches, and have the habit of lying on one side. The posterior edges of the shell valves, where the two

slender siphon tubes emerge, are bent upward. The tubes of the siphon (*es* and *is*) are independent of each other throughout, and their bases lie in a special sheath-like space of the mantle. At the base of the incurrent, a special muscle (*mm*) is developed in the mantle and is attached to both shell valves. The opening from the cloaca into the excurrent siphon is small and is situated on the dorsal side of the tube. Rectum (*r*) and both siphons lie to the right of the median plane of the body. Lying nearly in front of the opening of the incurrent siphon are thick folds of the mantle wall (*mf*) with serrated edges. The function of these was not determined. The crystalline style has a cap-like structure which fits into a depression of the stomach wall, as in *Schizotherus*.

Figure 33 shows that collections of the outer surface of the outer demibranch (*og*) are passed on to the surface of the inner demibranch, and are borne to its margin, as in several other forms. The attachment of the inner lamella of this demibranch to the visceral mass is so near the lower margin that the organ may hardly be said to hang free. The ciliation of the palps is the usual one, and visceral mass currents, instead of being directed backward, carry material forward to the dorsal margin of the posterior or inner palp. There are narrow tracts on the folded regions of the palps, directed both dorsalward and ventralward, but from lack of time and facilities when the form was examined, their precise situation in reference to folds and grooves could not be determined. Mantle currents were of the usual sort.

So far as these observations have gone, the *Macomas* are entirely unique in that the digestive tract, from esophagus to rectum, is usually packed full of sand. Not only so, but in most cases large quantities were found in the mantle chamber of *M. secta* and even the siphon tubes bore much of it. The form seems to have the earthworm habit of utilizing digestible material that happens to be present in large quantities of ingested soil, and it is of most striking interest that the outgoing tracts of palps and mantle are as well marked as in other forms; and, after cleaning the mantle chamber and using a smaller quantity of sand than that originally present, much of this is sent back

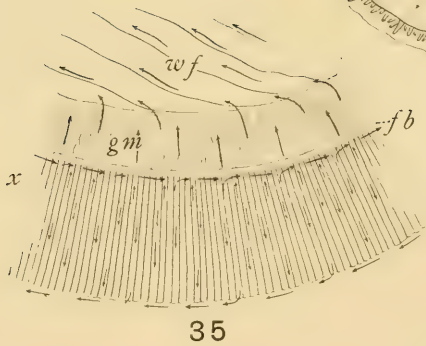
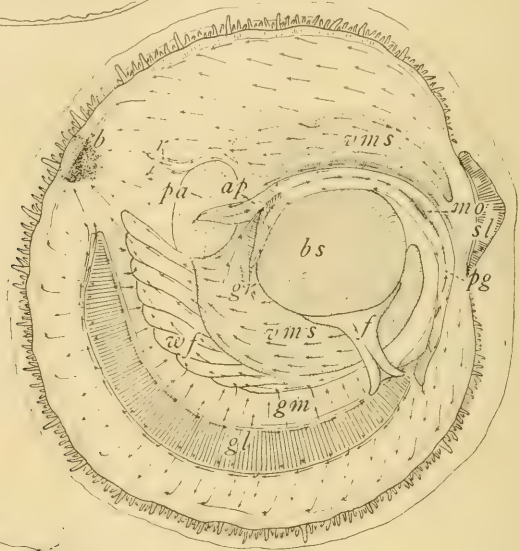
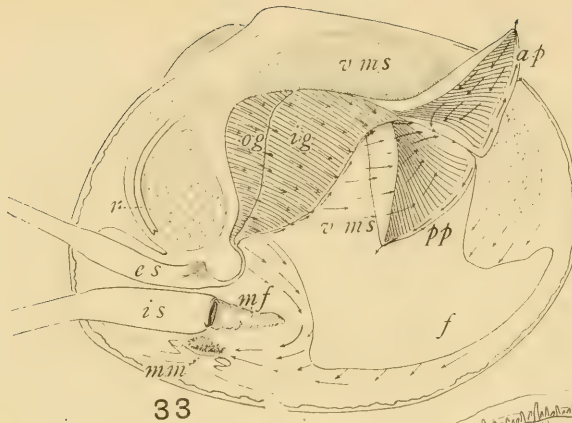


Fig. 33 *Macoma secta*; *ap*, anterior palp; *es*, excurrent siphon opening; *f*, foot; *ig*, inner demibranch of gill; *is*, incurrent siphon opening; *mf*, fold of mantle; *mm*, mantle muscle; *og*, outer demibranch of gill; *pp*, posterior palp; *r*, rectum; *vms*, visceral mass.

Fig. 34 *Monia machrochisma*; *b*, point on mantle edge where waste is collected; *bs*, byssus; *gl*, gill of left side; *gm*, suspending membrane of gill; *gr*, gill of right side; *mo*, mouth; *pa*, posterior adductor; *pg*, proximal oral groove; *sl*, shell ligament; *wf*, white folds of mantle; other letters as in figure 33.

Fig. 35 *Monia machrochisma*; gill ciliation; *fb*, free base of gill lamella; *gm*, suspending membrane of gill; *wf*, white folds of mantle; *x*, line on gill surface from which currents diverge.

and collected at the base of the incurrent siphon. Some of the sand in the mantle chamber is free, but much of it is cemented together by mucus from mantle, gills, and palps, and certainly the mechanism exists which may carry it all out of the body. Nevertheless, freshly opened specimens show so much sand covering all organs in the mantle chamber, that the outgoing tracts, if operating as in other forms, would prevent any material whatever from entering the mouth. It is impossible to believe that there has been any error in interpreting the outgoing tracts of lamellibranchs as mechanisms adapted for cleaning the mantle chamber of an excess of material which is usually undigestible and undesirable when the water is muddy, the object being to prevent its entrance into the digestive tract. Yet in *Macoma* enormous quantities of silt and sand do enter the digestive tract, and the failure of the outgoing system to operate as in other forms is as yet without explanation. It is a problem worthy of all the time that may be needed for its solution.

The tremendous power of cilia was especially well observed here, though perhaps really not greater than in other bivalves. The gills of *Macoma*, covered with a layer of sand a millimeter or more in depth, completely cleared themselves in the course of two minutes.

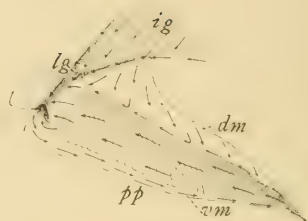
Monia machrochisma Desh

The rounded shells of the specimens studied at Puget Sound were more than 7 cm. in diameter. The form is particularly interesting on account of the extreme distortion and asymmetry of the soft parts of the body, and the shifting of several organs from their original positions. Probably on account of this, there have occurred reversals of some cilia currents from the usual course and the addition of others not found elsewhere, unless perhaps in *Anomia*, which has not been studied.

The left valve, as in *Anomia*, and to a less degree in *Ostrea*, is large and saucer-like, while the right is smaller and flat, and contains a deep bay through which the attaching byssus is extended. Figure 34 represents the soft parts, except the gills of the right side, when the right valve is removed. The region



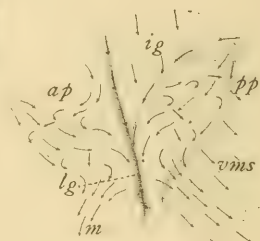
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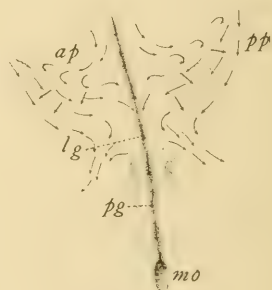
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of the mouth and palps of the left side have been twisted to the right, so that the former (*m*) opens toward the observer, and the entire extent of oral grooves (*og*) is seen at once. The foot (*f*), instead of being united with the visceral mass (*vms*) lies to the right of it, and is attached to the under side of the byssus (*bs*). There is a deep groove along its right side. If this is a byssus groove, the right is morphologically its ventral side. The gill of the left side (*gl*) is removed some distance from the visceral mass and is suspended by a membrane from the mantle wall. Just dorsal to the line of attachment, the mantle is thickened into very large, white folds (*wf*), structures not seen elsewhere, and perhaps containing extensions of the sexual mass. Around the edge of the mantle, a wide, thin fold arises perpendicularly from it, and extends inward, precisely as in *Pecten*.

Cills. The ciliation of the gill (fig. 34, *gl*) is different, in many respects, from that of all other gills examined. Figure 35 represents an enlarged portion of the inner left demibranch, showing its inner lamella, the suspending membrane (*gm*), and the white folds of the mantle (*wf*). The ciliation of all lamellae is like this. The thickened base of the lamella (*fb*) is free. Running parallel to this, there seems to be a line of division (dotted line *x*) of cilia streams on the lamella, material coming to its surface between this region and the base, being carried to the base and forward to the free ends of the palps. If in small

Fig. 36 *Tagelus californianus*; *ap*, anterior palp; *b*, bay of mantle; *f*, foot; *ig*, inner demibranch of gill; *m*, mantle wall; *pp*, posterior palp; *s*, siphons.

Fig. 37 *Tagelus californianus*; detail of palp ciliation; *dm*, dorsal margin of palp; *lg*, lateral oral groove; *vm*, ventral margin of palp; other letters as in figure 36.

Fig. 38 *Cardium corbis*; *dj*, distal oral groove; *og*, outer demibranch of gill; other letters as in figures 36 and 37.

Fig. 39 *Chama exogyra*; *aa*, anterior adductor; *b*, bay of mantle; *es*, excurrent tube of siphon; *f*, foot; *fo*, foot opening of mantle; *ig*, inner demibranch of gill; *is*, incurrent tube of siphon; *m*, mantle wall; *og*, outer demibranch of gill; *p*, anterior palp; *pa*, posterior adductor; *sl*, shell ligament; *vms*, visceral mass.

Fig. 40 *Chama exogyra*; detail of ciliation of gills, palps and visceral mass; *ap*, anterior palp; *lg*, lateral oral groove; *pp*, posterior palp; other letters as in figure 30.

Fig. 41 *Chama exogyra*; *lg*, lateral oral groove; *pg*, proximal oral groove; *mo*, mouth.

amount, it then proceeds along the extensive proximal oral groove and into the mouth. Below the line *x*, material is carried to the demibranch margin, and then *posteriorly* in its groove (fig. 34) to be cast off on to the mantle. This is the only species examined (except *Yoldia limatula* and *Pecten*, which possess mechanisms quite different) in which any part of the gill collections is carried away from the palps on outgoing tracts. Apparently the narrow strips at the bases of the lamellae collect sufficient food; but even their collections may be lost before reaching the palps, for if a large quantity of material is placed on them, some of it is apt to touch the suspending membrane (*gm*), in which case all will be pulled on to it and carried backward over the white folds of the mantle. In no other case has a more furious ciliary action been observed than on the gills and mantle of this form.

The anterior end of the gill of the right side is shown at *gr*. From this end, the gill bends downward, slightly forward, and then posteriorly in a curve parallel with that of the left side. It is suspended from the mantle, and its ciliation is like that of the left side.

Palps. The entire extent of these organs is shown in figure 34, and their chief cilia tracts are those of other bivalves, as indicated. Time was lacking to determine the presence or absence of the fine dorsally and ventrally directed tracts on the folds.

Visceral mass. The ciliation of the extensive visceral mass is indicated in figure 34, and requires no comment.

Mantle. All material collected or transported by the mantle is brought to a point, *b*, on the posterior edge, being held in place by the free fold until a large ball is formed. This is thrown out by a sudden contraction of the adductor which closes the valves. A very active special groove of the mantle parallels the oral groove anteriorly and dorsally. It is an outgoing tract, directing its stream posteriorly.

Tagelus californianus Conrad

The specimen examined was from San Diego Bay. Figures 36 and 37 show the ciliation to be the usual one in most respects. Currents on all gill faces are to the margins and forward. On

the visceral mass (not shown in the drawing) material is carried posteriorly, and cast off into the mantle chamber. The mantle ciliation is to the ventral edge and backward to the bay, *b*, below the incurrent tube of the siphon.

Figure 37 shows the anterior edge of the inner demibranch inserted far down into the lateral oral groove (*lg*), and united by conrescence along the center of its bottom. Streams from the outer demibranch come down the oral canal on both sides of this attached gill margin. The figure shows the abrupt turn of the lateral into the proximal part of the oral groove, which leads inward toward the mouth. There is a sharply defined current on the dorsal margin of each palp close to the upper ends of the folds, leading into the lateral oral groove.

Cardium corbis Martyn

Specimens were examined at Orcas Island, in Puget Sound. This *Cardium* is found in shallow water, only partially buried in the sand, so that unusually strong tide currents or waves throw individuals out on the surface. By means of the powerful foot, burrowing is easily accomplished.

Gills. The outer demibranch (fig. 38, *og*) is relatively small, and covers but a small portion of the inner (*ig*). Between the lines of their origin from the visceral mass, there is a considerable space (bounded by dotted lines in the figure). There is no groove on the rounded free edge of the outer demibranch, and collections on its outer surface are carried downward, around the margin, and upward on its inner face, to be taken by the broad ciliated tract on the wall of the visceral mass between the gills, to which reference has been made. Here they are carried forward, and then ventralward in the long distal portion of the oral groove, parallel to the anterior edge of the inner demibranch, then along the lateral part of the groove on its way toward the mouth.

The inner demibranch (*ig*) is attached to the posterior palp, as shown in the figure, but this does not modify the course taken by collected material, which is the usual one—to the marginal

groove on both lamellae, then forward, to be transferred to palp surfaces.

Palps. The organs are large and the ciliation of margins and folds, as indicated in the drawing, is of the usual character. It was in the Atlantic *Cardium mortonii* that the cilia tracts in the grooves between folds were first seen. There, and in *C. corbis*, the folds lift to expose the grooves when much material is present on the palps, and this, being swiftly drawn into them, is carried to the outgoing tract of the ventral margin. Attention should be called to the fact that the lateral part of the oral groove (*lg*), shown in this and many other figures, may experimentally be filled to overflowing with material, since currents are directed into it from above and from the sides. All this material will be moved downward, but, on reaching a point indicated by the line from reference letters *lg*, it encounters a powerful ciliation on the groove walls directed outward to the ventral palp margin. This outgoing ciliation extends so far into the groove at this point that only *small quantities* of material can continue in it toward the mouth. It is the very narrow gateway into the last and narrowest path to the mouth, the proximal oral groove, and is present in all cases in which the oral groove is extended upward on the sides of the body between the fused palps. Unless the sand eating *Macomas*, in which the mouth region was not carefully studied, are excepted, apparently in all cases only a very narrow and attenuated stream of material can enter the mouth.

Visceral mass. The ciliation is to the posterior wall, from which material falls to the mantle.

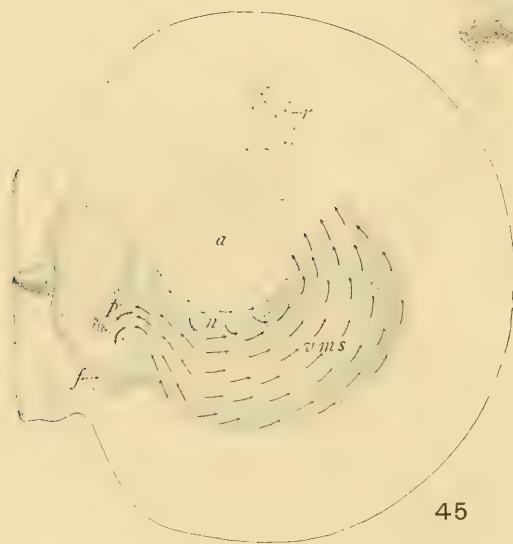
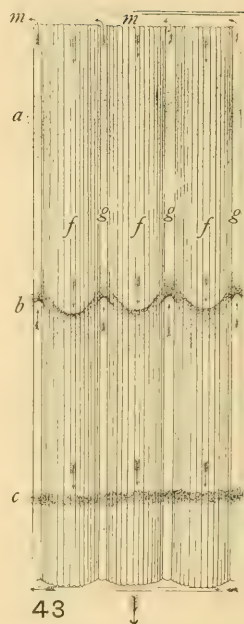
Mantle. Mantle collections are moved to a line parallel with its edge, then backward near the base of the incurrent siphon, where, over a small area, they are forced between the mantle edges to the exterior, instead of being collected and expelled periodically through the siphon. This is the usual procedure in forms with free mantle margins, and which are not completely buried in a burrow.

Chama exogyra Conrad

This extremely interesting attached form was studied at San Diego Bay. Figure 39 represents the left side after the removal of the flat, lid-like left valve and the left mantle fold. Like other attached species, outline and general form vary in conformity with the shapes of objects to which the creatures adhere. There has apparently been a contra-clockwise torsion of the body resulting in a great shifting of several organs. Usually, in forms in which the anteroposterior axis of the visceral mass is moved into line with the hinge of the shell, the anterior adductor diminishes in size or disappears (*Ostrea*, etc.) the posterior muscle meanwhile increasing in size, since it must perform the function of closing the valves. In this case, however, the anterior adductor (*aa*) has moved ventralward and backward, in reference to the axis of the visceral mass; the posterior (*pa*) dorsalward and forward, the anterior becoming the larger of the two. The rudimentary foot (*f*) has moved from an anterior to an extreme posterior position on the visceral mass, and the foot opening on the mantle edge (*fo*) has been shifted backward at the same time. Mouth and palps also have moved ventralward to some extent.

Gills. The ciliation of these organs is much like that of *Cardium*. The general current is downward on the outer lamella of the outer demibranch (*og*), around the edge of the narrow inner lamella, and forward along its base to the distal oral groove. Material is moved on both lamellae of the inner demibranch to a groove on the much thickened margin, and forward to the lateral oral groove. The relation of this demibranch to the lateral oral groove is shown in figure 40.

Palps. The palps present a condition not found in any other form examined, *in the reversal of the direction of the streams on their ventral margins*. The free ends of the organs project so far dorsalward that collections discharged from them would be in danger of being caught by the gill. Being reversed, the streams discharge on to the mantle from the anterior (fig. 40, *m*) and the visceral mass (*vms*) from the posterior palps. Palp folds



are large, and move in response to the stimulus of much material, so as to expose downwardly directed tracts in the grooves between them.

Mantle. On the upper mantle wall, there is a trend downward and backward, and all collections are brought to and accumulate at a point (*b*) under the base of the incurrent siphon (*s*, figure 39).

Pecten irradians and *P. tenuicostatus*

Pecten irradians is found from Cape Cod to Texas; *Pecten tenuicostatus* north of Cape Cod. The ciliation of organs of the body is essentially similar in the two species, and they may be considered together; minor differences in the course of cilia streams will be pointed out.

Gills. The organs are very extensive, and outer and inner demibranchs, on each side, are suspended by a thin, wide membrane. Figure 42 represents the outer demibranch of the left side of *P. tenuicostatus*, the gill membrane (*gm*) attached to the adductor muscle (*a*). The reference letter *g* is placed on the upper or basal edge of the outer lamella of the outer demibranch.

The mechanism for disposing of collections on all lamellae is astonishing in its efficiency, and its adaptation seems to be perfectly clear. A description of it was given in my "Shellfish industries" ('10) but perhaps should be repeated briefly here. The gill is plicated or folded; figure 43 gives a surface view of three of these plications, which are practically the same in all *Pectens*. Water, flowing between the filaments of the folds to the interior of the demibranch, brings suspended particles to the

Fig. 42 *Pecten tenuicostatus*; gill ciliation; *a*, adductor muscle; *g*, gill; *gm*, suspending membrane of gill; *x* and *y*, points between which material is received from the under side of the adductor muscle.

Fig. 43 *Pecten irradians*; gill mechanism for disposal of waste matter; *a*, carmine dropped on surface; *b*, same, rolled into a line; *c*, same, carried toward free margin; *f, f*, surfaces of folds; *g, g*, grooves between folds; *m, m*, tract leading toward palps.

Fig. 44 *Pecten irradians*; palp and gill ciliation; *ap*, anterior palp; *g*, gill seen from below; *mo*, mouth; *pp*, posterior palp.

Fig. 45 *Pecten tenuicostatus*; ciliation of visceral mass; *a*, adductor muscle; *f*, foot; *n*, nephridium; *p*, palp; *vms*, visceral mass; *r*, rectum.

exposed face of the lamella. As on all ciliated surfaces, these particles are caught and held by mucus. It is a point of prime importance that the touch of foreign substances causes the discharge of mucus, and in quantities proportionate to their volume. A few particles cause a slight local flow where they touch, and many cause a copious discharge—apparently in all cases just enough to hold the stimulating bodies, and prevent their escape. This seems to be true of all collecting surfaces, whether gills, visceral mass, or mantle. Cilia on filaments forming the bottoms and sides of the grooves between folds (fig. 43), cause a movement of mucus and suspended particles upward toward the base of the lamella (fig. 43, *mm* and fig. 42, *g*), where a tract leading to the palps is met. On the outer faces of the gill folds (*f, f*), however, cilia currents trend in the opposite direction, toward the edge of the demibranch. On this edge also there is a groove in which particles are carried toward the palps, as is usual in lamellibranch gills. Thus on every lamella there are two sets of currents, one directed toward its base, and the other toward its lower edge.

It is my conviction, based largely on the palp mechanism, that lamellibranchs (except, perhaps, the paradoxical, sand-eating *Macoma*) are able to take food into the digestive tract only when particles are brought to the gills a few at a time, and this belief is strengthened by the operation of the ciliary mechanism of the *Pecten* gill. Experimentally, it is easy to show that when a few scattered particles are brought to the gill surface the greater number of them will be drawn into the grooves, where, with the mucus holding them together, they are taken to the base of the lamella. From this region, they have repeatedly been seen to move to the oral groove and enter the mouth. The few particles falling on the folds are dragged off from them if there is a mucus connection with particles in the grooves; or, if not so connected, they move downward independently toward the free margin of the lamella. Being small in volume, they also are carried on this margin to the palps, and often on into the mouth. This I believe to be the mechanism in operation in feeding, when diatoms—probably seldom, if ever, extremely numerous—are

brought into the mantle chamber a few at a time in relatively clear water.

There is an entirely different disposition of particles when they are numerous, which is illustrated in figure 43. The stipples at the level *a* represent many particles, some having lodged in the grooves, others on the folds. They are numerous enough on the folds and in the grooves to be imbedded in a common mass of mucus, and instantly a strife begins between opposing streams for the control of the mass, as shown at *b*. The mucus being sufficient to hold together, it is evident that the downward thrust on the crests of the plicae tends to lift that part of the common mass resting in the grooves, and finally raises it until it loses contact with the groove cilia, which lash upward. Being drawn entirely out of the grooves, the whole is pushed rapidly downward on the crests of the folds to the free margin of the demibranch. Here there is a tendency to hold it in the groove leading to the palps, but the mass is usually too large, and falls off into the mantle chamber, where it will be picked up and carried out of the body. Between the two demibranchs, a tract leads forward to the palps (fig. 44, *g*).

This disposition of gill collections must inevitably occur when the entering water bears silt, or any other suspended particles, in a certain definite *quantity*. Silt, very fine sand, carmine grains, and even diatoms, have been used in the experiments, and in all cases, when their volume becomes great enough, the gill casts them off as described. With a little experience, while the introduced mass is settling in the water toward the gill face, it may quite accurately be predicted whether it will take the paths to the palps, or be gathered up and cast off the free margin into the mantle chamber. It was proved here, as well as in all other cases, that there is no recognition and selection of particles suitable for food in matter brought into the mantle chamber, but that the volume of material alone, of whatever nature, determined whether it would reach the mouth or not.

Muscular movements. Because of the length of this account, only brief reference has been made to muscular movements, common to all palps and gills, which aid the cilia in ridding

surfaces of large, or chemically irritating collections. It is an important function, and many observations have been made on it during the progress of this study; but it may only be stated that much material causes the gill grooves of *Pecten* to open wide,



Fig. 46 *Pecten tenuicostatus*; mantle ciliation; *a*, adductor muscle; *v*, *r*, vortices; between *x* and *y* material is moved to the surface of the adductor muscle.

Fig. 47 *Pecten irradians*; mantle ciliation; letters as in figure 46.

and then to close with so sudden a contraction that material is thrown out of them. Often this violent bending of filaments, which results in spreading open and then constricting the grooves,

occurs in about a second of time. The whole demibranch, also, may present a wavy surface, and sway, fanwise, toward the mantle and inward—apparently an adaptive movement, for material is sometimes directly transported from the outer demibranch to the mantle surface, and in any case, tends to be shaken off into the mantle chamber, so rapid is the swaying movement. Extensive movements of the gill of *Yoldia* have been described by Drew ('99) and the writer ('90) in which organs there are well developed muscles; but in the *Pecten* gill and others, also capable of extensive movements, such muscles are absent. Large quantities of material on the palps also cause the mouth to close tightly.

Suspending membrane. Currents on the suspending membrane in *P. tenuicostatus* are shown in figure 42. On the opposite side of the membrane the currents are practically the same, both carrying material to the free edge above, where it is cast off into the mantle chamber. Between the points *x* and *y* material is received, on this outer face of the membrane, from the under surface of the adductor muscle (*a*). Curiously enough, the muscle wall received this material from the mantle (between points *x* and *y* in figures 46 and 47).

Palps. Figure 44 represents the mouth and the highly modified palps of *P. irradians*, the anterior palp (*ap*) being folded forward. The anterior ends of both demibranchs of the left gill (*g*) lie well in between the palps, and the manner in which the various gill streams are continued into the oral groove is shown. Many times, very small quantities of material were seen to pass from gills to oral groove and into the mouth, while larger masses were drawn out of the groove and carried diagonally across the folds, being conducted to the mantle by the anterior, and to the visceral mass by the posterior palps. The margins (on which the reference letters are placed) are partially united to the mantle, in the case of the anterior, and to the visceral mass in the posterior palps, and material on mantle and visceral mass near the palps is transferred to them across their margins, and back to them again after crossing the folds. The almost uni-

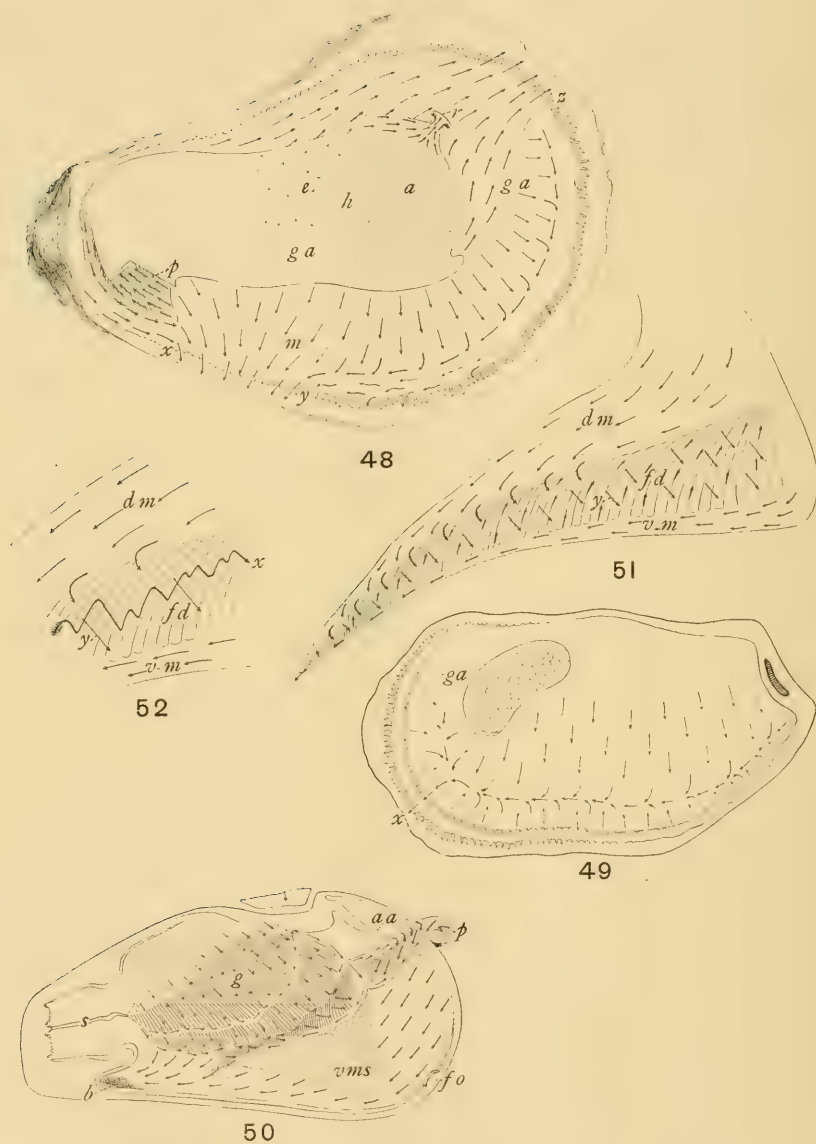


Fig. 48 *Ostrea virginica*; a, adductor muscle; e, union of mantle and epi-branchial chambers on the right side; ga, ga, lines of gill attachment; h, heart; m, mantle wall; p, inner palp; between x and y waste is expelled from mantle chamber.

versal posteriorly directed current along a plain ventral margin, seems not to be present.

Visceral mass. Currents on the visceral mass of *Pecten* are represented in figure 45. In the region of the foot (*f*) collections from a considerable area are carried forward to the posterior palp (*p*). On the remainder of the wall of the body, collections are carried posteriorly and thrown off into the mantle chamber along the side extending from the pointed posterior end to the adductor muscle (*a*). The ciliation is practically the same in *P. irradians*, except that, posteriorly, material is all cast from the pointed tip.

Mantle. It is shown in figure 46 (*P. tenuicostatus*) and 47 (*P. irradians*) that the greater part of the mantle collection is carried to a position above the rectum (*r*). A less amount, most of it gathered from the mantle edge, is carried to a corresponding position on the ventral side. At these points, in *P. tenuicostatus*, the material is forced into well defined vortices (*v,v*) where relatively large collections are made. These are discharged from the body by a sudden, but not complete closure of the valves. There are no vortices on the mantle of *P. irradians*, but at corresponding positions, currents carry material over the mantle edge, often aided by a closing of the valves. Between the points *x* and *y* on the posterior face of the adductor muscle, some material is deflected on to the muscle wall, from that to the suspending membrane of the gill, from whence it falls once more to the mantle, the adaptation in the course taken not being apparent.

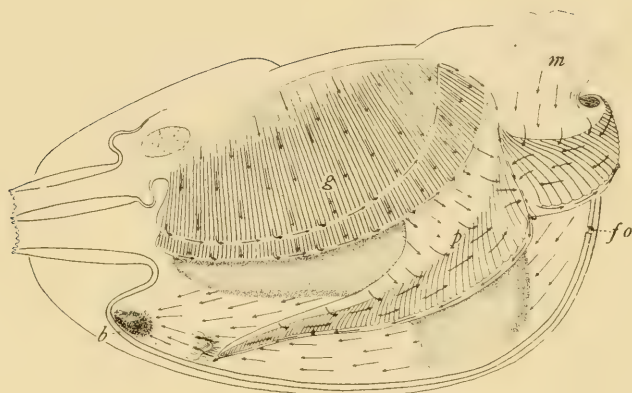
Ostrea virginica

Gills. On all gill lamellae, collections are carried to the margins, and moved in grooves toward the palps. At the base of each lamella there is also a tract carrying material forward.

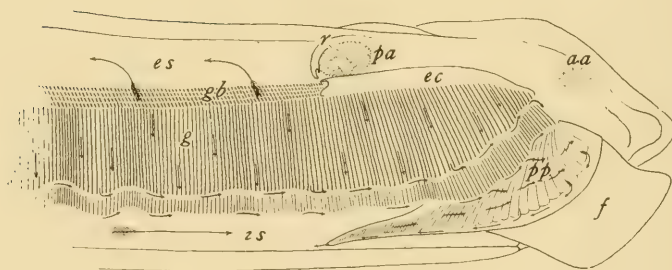
Fig. 49 *Ostrea lurida*; mantle ciliation; *ga*, line of gill attachment; *x*, a line over which waste is expelled.

Fig. 50 *Pholadidea penita*; *aa*, anterior adductor; *b*, bay of mantle; *fo*, foot opening through mantle; *g*, gill; *p*, anterior palp; *s*, siphon; *vms*, visceral mass.

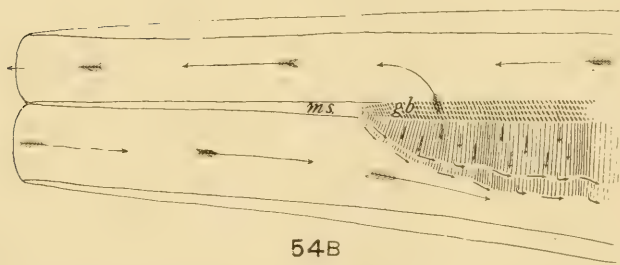
Figs. 51 and 52 *Pholadidea penita*; detail of palp ciliation; *dm*, dorsal margin of palp; *fd*, palp folds; *vm*, ventral palp margin; *x*, course taken by a small amount of material; *y*, ciliation across the folds.



53



54A



54B

Fig. 53 *Pholadidea ovoidea*; *b*, bay of mantle; *fo*, minute foot opening through mantle; *g*, gill; *m*, part of mantle thrown forward; *p*, posterior palp.

Fig. 54 a *Zirfaea gabbi*; *aa*, anterior adductor; *ec*, epibranchial chamber; *es*, excurrent tube of siphon; *f*, foot; *g*, gills; *gb*, bases of gills; *is*, incurrent tube of siphon; *pa*, posterior adductor; *pp*, posterior palp.

Fig. 54 b *Zirfaea gabbi*, posterior end of body; *gb*, bases of gills; *ms*, muscle septum between siphon tubes.

Palps. The ciliation of the thick, heavy palps presents the usual features. In figure 48 a part of the anterior palp of the left side has been removed (at *p*) and exposes a part of the folded surface of the posterior palp, and the primary course across folds toward the mouth. The ventral edge, with its backwardly directed current, is very narrow. The outer faces, or those without folds, are ciliated, and direct material backward to the tips.

Mantle. Posteriorly and below the line of gill attachment (*ga*), material is moved to a line lying parallel with the mantle edge, and then along this to a point *y*, between which and the point *x*, it is carried over the edge. Between *y* and *z* on the edge, there is a feeble ciliation directed outward over the edge. Posterior to the adductor muscle (*a*) and above the line of gill attachment, there is a feeble ciliation upward and backward, over the edge above the point *z*. There is here also a strong current of water from the gills, which takes the same course, and aids in sweeping outward feces from the rectum (*r*). The mantle and the greater part of the visceral mass wall are fused on the left side. On the right, there is a space (*e*) anterior to the adductor, where mantle and epibranchial chambers communicate. The mantle wall here, and also that of the visceral mass, possess a ciliation directed upward.

Figure 49 represents the mantle ciliation of *O. lurida* of the Pacific. At the point *x* is a narrow line over which collections pass from the mantle edge.

Pholadidea penita Conrad

Specimens were taken from San Diego Bay, California. The form is a true borer; it possesses an unpaired plate dorsal to the hinge.

Gills. Cilia currents on all lamellae are to the free edges of the demibranchs and forward (fig. 50). There is a current forward in the angle between demibranchs.

Palps. The lateral extension of these organs is great. The ventral margins are well defined, and those on the dorsal edges are exceptionally wide. The currents of the apposed faces are

represented in figures 51 and 52. In the former the general current on the folds is seen to be obliquely forward and markedly downward across them. Between the folds are dorsally directed currents which seem always to be exposed, not simply by the parting of folds in response to the stimulus of large quantities

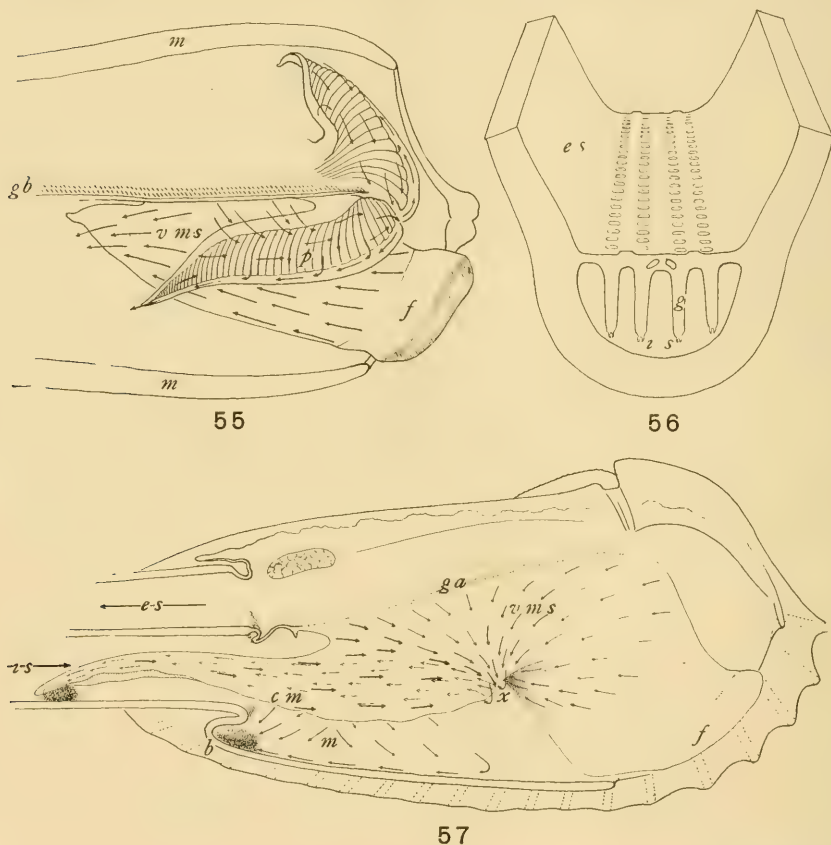


Fig. 55 *Zirfaea gabbi*; ciliation of palps and visceral mass; f, foot; gb, gill bases; m, mantle wall; p, posterior palps; vms, visceral mass.

Fig. 56 *Zirfaea gabbi*; section across siphon; es, excurrent tube of siphon cut open dorsally; g, gills; is, incurrent tube of siphon.

Fig. 57 *Barnea costata*; b, bay of mantle; cm, collecting membrane; es, excurrent tube of siphon; f, foot; ga, line of gill attachment; is, incurrent tube of siphon; m, mantle wall; vms, visceral mass; x, point at which material from visceral mass passes to collecting membrane.

of material, as in *Schizotherus* and others. The result is, as shown in figure 5², that large and small quantities of material alike are usually carried in a broken, zigzag course to the lateral portion of the oral groove, much as indicated by the long feathered arrow. Small masses in the lateral groove pass on toward the mouth, while larger quantities are invariably caught up out of it by cilia of the ventral margin, and conducted to the palp tip.

Visceral mass. The ventral portion of the visceral mass is shown in figure 50 (*vms*). The foot has disappeared—there are no remains of foot muscles—in mature individuals, though a small foot opening persists far forward in the fused mantle edge (*fo*). The crystalline style shows through the thin epidermis at the point of the mass. The walls of the upper portion of the mass are ciliated, and conduct material to its posterior side, where it is cast off into the mantle chamber in the usual manner.

Mantle. In the same figure, the mantle is shown to present no unusual features.

Pholadidea ovoidea Conrad

Specimens were taken from burrows about four inches deep in extremely hard clay, between tide lines on Puget Sound. Anatomically the form is very similar to *P. penita*, but unimportant differences are shown in figure 53. The lateral parts of the palps are enormously enlarged, especially the dorsal margins, which are fused extensively with the mantle (*m*) in the case of the anterior, and with the visceral mass in the posterior palps. The visceral mass is relatively more extensive than in the preceding species, and of a different form ventralward. The foot is entirely absent, the sexual mass entirely filling the foot-like projection of the visceral mass; but a minute foot pore (*fo*) opens through the mantle edge far forward. There is a small forwardly projecting fold on the left mantle wall under the incurrent siphon. Whatever it may be, it has no relation to the ciliary mechanism.

The ciliation of organs is like that of *P. penita* except on the palps, where the general current is directly across the folds

toward the lateral oral groove; and no dorsally or ventrally directed currents were found between folds. Only two specimens were available for examination, however, and it may be that such tracts exist.

Zirfaea gabbi Tryon

The shell of one specimen of this Pholad taken from Puget Sound measured 12 cm. or nearly five inches in length. The animals were dug with a pick from blue clay, nearly as hard as rock, and the burrows of larger individuals were about 20 inches in depth. Siphon and gill development here, and in some other members of the family, are very extraordinary, as shown in figure 54. The mantle chamber cannot be distinguished from the incurrent siphon tube, the whole mantle being extended posteriorly without the usual constriction of the siphon base. Instead of ending posteriorly at the base of siphon tubes, as is usual, the gills extend backward, in an uncontracted siphon, for nearly two-thirds of its extent, attaining an actual length, in large individuals, of a foot or more. The tubes of the distal third of the siphon are separated by a muscular septum (*ms*), the dividing wall of the remainder being formed by the gills which are united on the median line and joined laterally to the siphon wall, as shown in figure 56. In this figure the excurrent siphon is cut and opened dorsally. Possibly the distal region only should be regarded as true siphon, but a strong argument could be formulated against such a view. Mantle and siphon walls are of great thickness. There is a piston-like foot which may in some way aid in boring; but it may be that the great muscular development of the siphon enables the animal to impart a drilling motion to the anterior part of the body when the structure fits tightly in the burrow. It would be interesting to know how, during its growth, this and other borers manage to deepen and enlarge their burrows.

Gills. On all lamellae of these enormously extensive collectors, currents trend ventrally to the margins, where material is received into grooves. Apparently the conduct of material

over the distance of a foot—as some of it must be carried—in the midst of the rushing stream of the incurrent siphon, would be attended by so much uncertainty in an open groove, that completely covered passages have been developed on the edges of the demibranchs, which assure the delivery of the gill collections to the palps. When material from the lamella reaches the closed groove, its walls part, admit the collection, and close over it, if its volume is not too great.

Palps. These organs are relatively large, and bear large folds. The anterior palp is not shown in figure 54. In figure 55, the mantle has been cut ventrally, the right side being lifted to show the attachment of the anterior palp to it, as well as the short line of union between posterior palp (*p*) and visceral mass below the line of gill attachment (*gb*). The ciliation of folds and margins is beautifully clear, and is found to be essentially like that of the *Schizotherus* palp, except that no dorsally directed currents were observed anywhere on the crests of the folds.

Visceral mass. Posteriorly the visceral mass is extended into a point, just below which its collections are cast into the mantle chamber, as shown in figure 55.

Mantle. No case has been observed among lamellibranchs, in which the inner walls of the siphon tubes were ciliated. There is in *Zirfaea* a downwardly directed ciliation on the mantle walls opposite the visceral mass, that trends posteriorly on its ventral surface. There is no bay beneath the siphon base, as in other forms, and apparently no point where a collection is made. Material simply gathers on the ventral mantle wall, and is no doubt discharged through the ventral siphon from time to time, as a result of the contraction of the adductor muscles. The mantle streams, in the forms examined, were very feeble.

Barnea costata Lin

This species, studied on the coast of Louisiana near the mouth of the Calcasieu river, is remarkable in having developed an organ, the function of which is to extend and perfect the ciliary mechanism for freeing the body of undesirable foreign matter.

Excepting this organ, the ciliation of the body conforms, in general, to that usually found in bivalves.

Proceeding at once to a description of what may be called for lack of a better name, the collecting membrane of the visceral mass, it will be noticed that the thin, filmy structure (fig. 57, *cm*) is an outgrowth of the posterior wall of the body (*vms*), and projects into the mantle chamber. It may be retracted close to its base, or extended backward for a great distance, when fully performing its functions being thrust into the incurrent siphon tube. Because of its great power of extension, it does not seem impossible that its end may sometimes be projected entirely through the siphon tube. It tapers from base to tip, and has the form of an inverted trough. In the figure, cilia currents on the extensive collecting area of the visceral mass, below the line of gill attachment (*ga*), converge on each side of the body to the base of the membrane, on its ventral edges, at the points *x*, right and left. An attempt has also been made to show that the outer or upper convex surface of the membrane is ciliated, and carries material to the same regions. Here all collections pass to the under, concave, surface of the collecting membrane, and are carried swiftly backward, directly in the face of the incoming stream of water, to the tip of the membrane, as represented by dotted arrows. Here they are held—the tip usually resting on the siphon wall—until a ball of considerable size has been collected. This, and the mantle collection at *b* are discharged on contraction of the adductor muscles.

Specimens in which this membrane was found, were taken from waters which frequently were almost thick with silt for many hours at a time, but it was observed that mud was never so abundant as to cause the creatures to withdraw their siphons, and so prevent its entrance to the mantle chamber. This was clearly seen at a stage of the ebb tide when only a film of water covered the flats in which individuals were numerous. The presumption is that, under such conditions, the ordinary means of removing mud were insufficient, and that this collecting membrane was developed as an aid. At any rate, that mud

removal is its function, is not to be questioned. What seems to be an homologous structure, was found in *B. pacifica*.

Barnea pacifica Dall.

This beautiful form was studied at San Diego Bay, and was taken from stiff clay, the burrows being four or five inches in depth. In relation of siphon to body, it much resembles *Zirfaea*, previously described. As in that form, the gills extend posteriorly far into the siphon (fig. 58), there forming the septum between incurrent and excurrent tubes.

Gills. Currents on all lamellae are downward to demibranch margins, where, as in *Zirfaea*, the groove leading to the palps possesses walls so high that they arch over and convert it into a closed tube, opening to receive and discharge collections (fig. 59). There are anteriorly directed streams between the bases of the demibranchs.

Palps. Here, again, these organs are relatively extensive. The ciliation of folds and margins is precisely similar to that of *Pholadidea penita*, except that here a very narrow tract, lying along the upper ends of the folds, lashes toward the oral groove, but is so narrow that collections from the dorsal margin cross it on the way to the folds, with little influence from it. It is safe to say that there are no ventrally directed tracts on the palp folds.

Visceral mass. Figure 60 represents the visceral mass from the right side, after mantle, palps, and gill have been removed. Ventralward there is a disk which contains some muscle tissue, but the sexual mass extends to the disk surface, and no foot remains, unless a few scattered muscles are the vestiges of it. Figure 61 is a ventral view of the visceral mass. In the last three figures there is shown a thin, nearly transparent structure (*cm*) with a slight concavity on its ventral surface, which is evidently the collecting membrane so greatly developed in *B. costata*. When a comparison is made with figure 57, the course taken here by cilia currents is very suggestive. It will be observed that these currents are directed to a region on the side, near the middle of the body, whence they course to the

ventral wall, and then posteriorly until they reach the end of the collecting membrane. Without extending itself, this membrane lies well within the base of the incurrent siphon, because of the

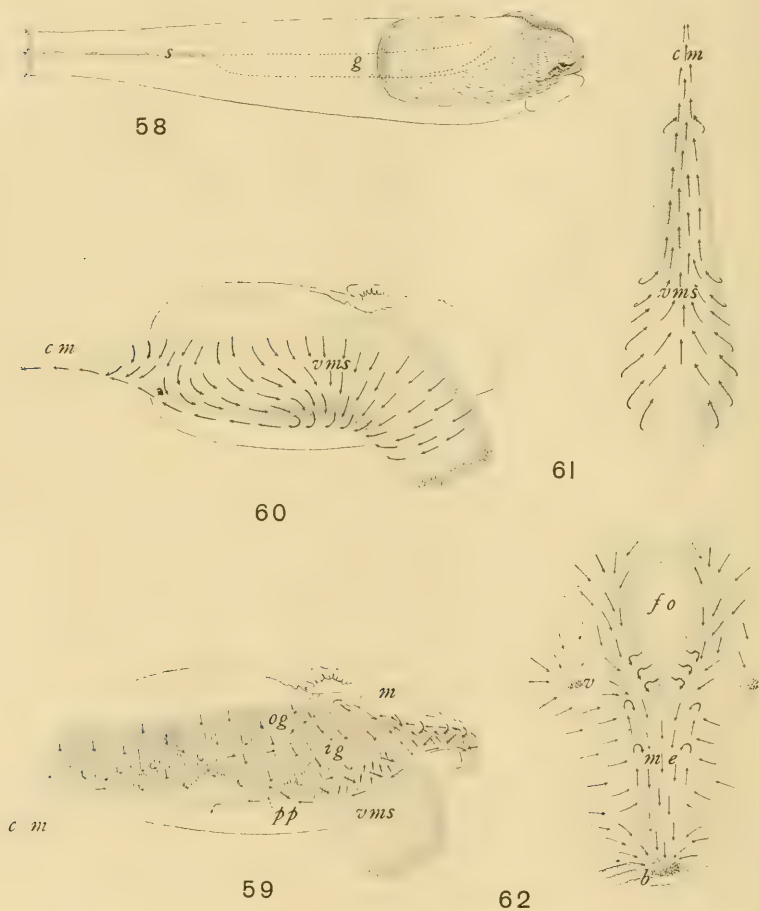


Fig. 58 *Barnea pacifica*; *g*, position of gills; *s*, siphon.

Fig. 59 *Barnea pacifica*; *cm*, collecting membrane; *ig*, inner demibranch of gill; *m*, portion of mantle thrown forward; *og*, outer demibranch of gill; *pp*, posterior palp; *vms*, visceral mass.

Fig. 60 *Barnea pacifica*; ciliation of visceral mass; letters as in figure 59

Fig. 61 *Barnea pacifica*; ventral view of visceral mass ciliation.

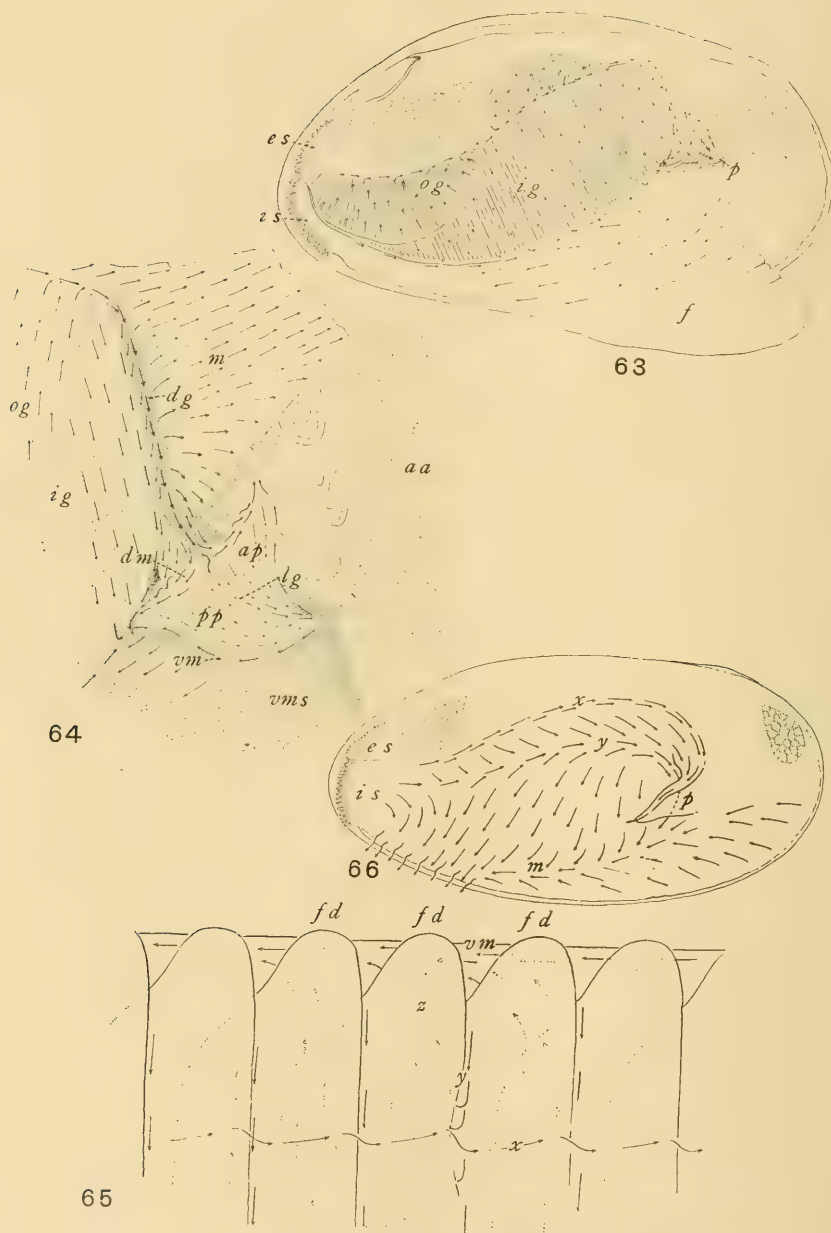
Fig. 62 *Barnea pacifica*; ciliation of mantle edges seen from above; *b*, bay of mantle; *fo*, foot opening of mantle; *me*, fused mantle edges; *v*, vortex.

backward prolongation of the visceral mass. Whether collections accumulate on its ventral surface or not, could not be determined. Because the course of cilia currents over the entire visceral mass is so much like that of *B. costata*, it would seem that there is here a much degenerated collecting membrane, which formerly may have been much more extensive.

Mantle. The walls of the mantle chamber, unlike those of *Zirfaea*, may be distinguished from siphon walls, because they are very thin, while the siphon walls are muscular. Currents are directed ventralward from the sides to the fused ventral margins. The details of currents in this region are shown in figure 62 which represents a view of the fused ventral mantle edges seen from above. The foot opening (*fo*), through which the disk-like end of the visceral mass may protrude slightly, is still large. Some of the material brought from the side walls of the mantle, pauses, and may collect in small balls at two points (*v*) which suggest the vortices of *Mya*. There seems to be no whirling movement here, and collections are sooner or later moved on to the thickened and fused edges. At several places, as shown by the arrows, they pass on to a median, broad, slightly depressed tract (*me*) and are carried swiftly backward to a point, *b*, corresponding in position to the bay at the base of the incurrent siphon, which has been shown to be so generally present on the lamellibranch mantle.

Unio complanatus Solander

Gills. Specimens were from a lake in western Massachusetts. On both lamellae of the outer demibranch (fig. 63, *og*) collections are carried to the base, where, on each side, they are moved anteriorly, along narrow lines, to the distal oral groove, just in front of, and parallel with, the anterior margin of the inner demibranch (fig. 64, *dg*). Unless the material is very small in amount, it touches the anterior wall of this groove made by the mantle (*m*), and is lifted out and carried away by the mantle cilia. On both lamellae of the inner demibranch, material is carried to the free margin, and then forward in its groove to a



point just posterior to the palps (below the reference letters *dm*) where it collects, if not at once transferred to the apposed palp faces. Collections are also brought downward to this point, along the demibranch edge lying parallel with the distal oral groove. It is probable that in all bivalves, the palps may be withdrawn from contact with this demibranch edge, so that accumulations on the margin become great enough to fall of their weight to the mantle or visceral mass walls.

Palps. *Unio* is one of those forms in which the dorsal edges of the lateral extensions of the palps are united so as to bring the sets of folds close together, leaving but a very narrow tract (the lateral oral groove) between them (fig. 64, *lg*). Where the distal joins the lateral part of the oral groove, the dorsal margins make a short trough (to the left of reference letters *ap*). The ventral palp margins (*vm*) in the *Unios* and *Anodons* are narrow, as are the folds, and observation of their ciliation is difficult. The wide dorsal margin of the posterior palp receives much material collected by the visceral mass in its vicinity; that of the anterior, in the same way, receiving mantle collections on its inner, and also on its outer face from contact with the mantle. These points are not well shown in the figure. The outer faces of both palps are ciliated, and bear material to the free dorsal margins, where it comes over on to the inner faces. None of it is moved to the folds, but all is carried to the free tips and thrown off.

The general ciliation on palp folds is nearly straight across them, forward. The details of the ciliation on the folds are

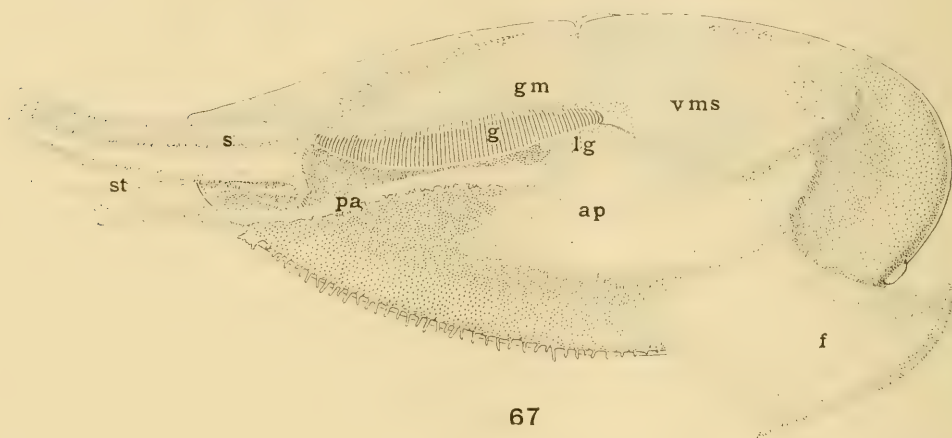
Fig. 63 *Unio complanatus*; *es*, excurrent opening of siphon; *f*, foot; *ig*, inner demibranch of gill; *is*, incurrent opening of siphon; *og*, outer demibranch of gill; *p*, palps.

Fig. 64 *Unio complanatus*; ciliation of palp region; *aa*, anterior adductor; *ap*, anterior palp; *dg*, distal oral groove; *dm*, dorsal palp membrane; *ig*, inner demibranch of gill; *lg*, lateral oral groove; *m*, mantle, *og*, demibranch of gill; *pp*, posterior palp; *vm*, ventral palp margin; *vms*, visceral mass.

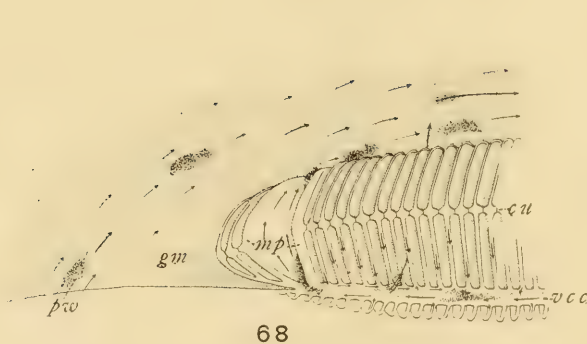
Fig. 65 *Unio complanatus*; detail of ciliation of palp folds; *fd*, *fd*, *fd*, palp folds; *vm*, ventral palp margin; *x*, direction of ciliation across folds; *y*, separation of folds to expose deep cilia tract; *z*, course of particles over deep tract.

Fig. 66 *Anodon* sp.; mantle ciliation; *es*, excurrent opening of siphon; *is*, incurrent opening of siphon; *m*, mantle wall; *p*, palp.

shown in figure 65, which represents the ends of several folds (*fd,fd*) near the ventral margin (*vm*). While the lower ends of the folds project for some distance ventralward, they are raised



67



68



69

Fig. 67 *Yoldia limatula*; *ap*, anterior palp; *f*, foot; *g*, gill; *gm*, suspensing membrane of gill; *lg*, extension of lateral oral groove; *pa*, palp appendage; *s*, siphon; *st*, siphonal tentacle; *vms*, visceral mass.

Fig. 68 *Yoldia limatula*; ciliation of gill and suspensing membrane; *cu*, line of ciliary unions; *gm*, suspensing membrane of gill; *mp*, modified plates of gills; *pw*, waste from palps; *vcc*, ventral ciliated canal of gill.

Fig. 69 *Yoldia limatula*; ciliation of ventral surface of gill; Letters as in figure 68.

above the margin, not touching and interfering with its current. The folds lie over anteriorly—toward the right of the figure—each covering a part of the base of the one in front of it, in the usual manner. The line of arrows *x* represents the general course taken by particles across the folds toward the mouth. On each fold, close to the edge of the fold behind it (as just above the letter *y*) there are very narrow tracts, the cilia on which direct currents dorsalward toward the lateral oral groove. These have some influence on material crossing the folds, tending to keep it away from the ventral margin, on which it would be carried away from the mouth. When much material appears on the folds, they lift here and there (*y*), so that it is caught quickly and certainly by cilia deeper in the grooves, and, as represented by the dotted arrows, is carried to the outgoing stream on the ventral margin, precisely as in *Schizothreus*, *Cardium*, and others.

Mantle. Figure 66 represents the ciliation of an *Anodon*, which is in most respects similar to that of *Unio*. In both, material is carried out over the margin below the lower siphonal opening (*is*), as is usual in forms not completely buried.

Yoldia limatula

If this much discussed genus is properly placed among the most archaic of living lamellibranchs, this representative of it certainly possesses the most extraordinarily complex set of ciliary mechanisms observed in the group. It lives in soft mud, and, as shown by the stomach contents, allows relatively large quantities of indigestible material to pass into the digestive tract, though the relative volume of diatoms found, is very much greater than in *Macoma*. *Yoldia* does not, by any means, feed simply on what it is able to digest out of the mud of the bottom in which it lives, for diatom shells would not in that case form so large a part of the stomach contents; and several very elaborate and effective mechanisms exist, the function of which is to clear the organs exposed in the mantle chamber of objectionable material brought by the incurrent stream.

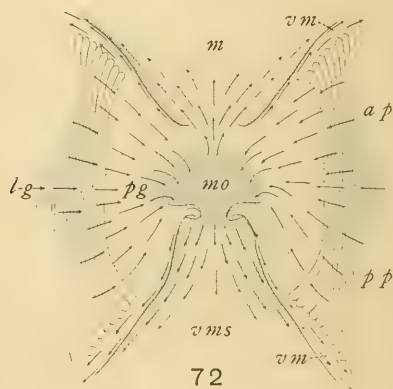
In order to understand the operation of these mechanisms, it is necessary to have a clear notion of the relative positions of



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several organs bearing ciliated surfaces. In figure 67 the reference letters *ap* are placed on the anterior or outer palp, the edge of the posterior, which it overlies, appearing beyond its back margin. Arising from about the middle of the dorsal margin of the outer palp, is a long appendage (*pa*) which may be extended beyond the shell margin for a distance greater than the length of the shell. Above and behind the palps hang the gills (*g*) that are suspended from above by a membrane (*gm*). It is with the relative positions of gills and palps that we are here concerned. The two palps, on either side of the body, are united along their dorsal edges, upward from the point where the appendage arises from the anterior or outer, and along this line of union is a deep groove, an extension of the lateral oral groove, which lies hidden between the palps below the region of the visceral mass (*vms*). This extension of the groove lies beneath the reference letters *lg* in figure 67 and is shown in figure 70, at *lg*. It is often placed in such a position that it touches the lower surface of the anterior end of the gill, though in the first figure, the gill is raised somewhat above it, and in the second (fig. 70) this entire dorsal region of the palps is pulled downward, their free ends being spread apart in order to expose all of their ciliated surfaces. In its normal position, then, this extension of the oral groove (*lg*) lies against, or close to, the ventral surface of the gill, the reason being, as will be shown, that, from time to time it receives the gill collections.

The anatomy of the gill, first described in detail by the writer, is well known, as it has been studied by several observers, but one important structural modification has been overlooked. I know of but one brief reference to it—that found in Drew's paper on *Yoldia* ('99) the reference being:—"With the exception

Fig. 70 *Yoldia limatula*; detail of palp ciliation; *dm*, *dm*, dorsal margins of palps; *g*, gill; *gm*, suspending membrane of gill; *lg*, extension of lateral oral groove; *paa*, palp appendage; *vm*, ventral margin of palp; *vms*, visceral mass; *x*, notch in groove passing to mouth.

Fig. 71 *Yoldia limatula*; ciliation of outer surface of palp appendage.

Fig. 72 *Yoldia limatula*; ciliation of mouth region; *ap*, anterior palp; *lg*, lateral oral groove; *m*, mantle; *mo*, mouth; *pg*, proximal oral groove; *pp*, posterior palp; *vm*, *vm*, ventral margins of palp; *vms*, visceral mass.

of a few of the plates at the extreme anterior ends of the gills, *which are sometimes much distorted and swollen* [italics mine], all of the plates are alike in shape and structure."

The modified plates which Drew saw are always present, are two in number (the third and fourth in the series, though if this may possibly vary, such variation has not been seen) and are always found only on that side of the gill nearest the median plane of the body. They are modified to dispose of gill collections which are not to be sent to the palps, being one more of the several anatomical structures that are developed to aid the ciliary mechanisms. Figure 68 represents the anterior end of the right gill seen from the inner side, so as to show the modified plates (*mp*). Along the sides of the plates is the series of ciliated unions (*cu*) that I have described elsewhere. On the ventral surface of the gill, and between the two series of plates, is a broad ciliated canal (*vcc*). The edges of the plates above *cu* are not ciliated, but below this line is a full ciliation of the plate edges which drives water upward between them (see the long feathered arrow) into the epibranchial space, and carries particles brought to them very swiftly down to the ventral canal. Here the collections are carried forward, but are halted momentarily about opposite the fifteenth plate by a narrow, backwardly directed tract lying along the bases of the inner plates (see also fig. 69, a ventral view). Very small amounts seem to pass this point without interference. It is possible that the halt is made here in order to facilitate the transfer of material to the palps, the oral groove of which, at times, lies against this region of the gill. At any rate, if this transfer is not made, the material, after revolving a few times, continues on toward the modified plates. These, like all parts of gills and palps, as described by Drew and myself, are capable of extensive movements. They are never in close contact (they possess no ciliated disks on their apposed faces) but may spread wide apart. Their apposed faces are richly ciliated, and material brought by the ventral canal is seized and quickly forced through between them into the epibranchial space. Here, on the wall of the suspending membrane (*gm*), and aided, perhaps, by the outgoing stream of

water, they leave the body through the excurrent siphon. In no other gills that I know of except those of *Monia*, and *Pecten*, are there special means of conducting undesirable material to outgoing tracts. In other cases, collections may be of such volume that the marginal groove cannot hold them, and they fall into the mantle chamber of their own weight. It is the function of other gills simply to collect, and pass collections on to the palps on which it is determined whether they shall be continued on to the mouth, or to an outgoing tract; but here the gill possesses its own outgoing tract, which must inevitably be used unless contact is effected between gill and palp.

Palps. The huge lateral extensions of these organs are for some distance suspended from the overhanging digestive gland (fig. 67, *vms*). Behind the line of this attachment, and as far toward their free ends as the origin of the appendage, their dorsal margins continue to be united, and on the outside along the line of this union is the groove (fig. 70, *lg*) the function of which apparently is to receive the gill collections. This groove, so far as I know, has never previously been seen. From its position in reference to the ventral surface of the gill, from the direction of its cilia currents, and from the fact that its margins may convert it into a nearly closed tube, or open so fully as to expose it, I am convinced that it has been constructed to receive gill collections, though I have no record of having seen the transfer actually made.

Gill collections and the palps. Drew, from his study of the gills and palps, concluded that the former were not food collectors, as Mitsukuri ('81) had done, also. He says:

Experiments were tried to determine, if possible, the part taken by the gills in the collection of food. . . . No definite results were reached, but they were not observed actively engaged in collecting food. Considering the remarkable activity of the palps as collectors of food, such activity for the gills seems rather unnecessary, and it would also seem that the pumping action of the gills would seriously interfere with their normally performing such a function.

Drew had not seen the extension of what I have called the lateral oral groove, but I am puzzled to know how the tremendous

activity of the gill in collecting and moving forward suspended particles brought to it in the water, could have escaped his notice, the whole process being precisely like the food collection of other lamellibranch gills. The pumping action of the gills does not disturb small collections, and there is no reason for assuming that it would interfere with the transfer of food from gill to palp.

Collections of the palp appendage. In the quotation above, Drew refers to his own observation of collections made by the palp appendages, which I have confirmed many times. This structure (fig. 70, *pa*) is a trough, the convex surface normally opening ventralward when it is extended from the body, and allowed to rest on the surface of the mud. Here, as Drew describes it, great quantities of material, "foraminifers, ostrocods, and even small lamellibranchs and gastropods, together with the smaller forms and mud, are passed along the groove, finally between the palps, and so on into the mouth."

The relation of this groove of the appendages to other ciliated tracts—the oral, and several outgoing streams—is shown at the point *x* in figure 70. The letter is placed close to a narrow notch, bounded on either side by the outgoing tracts of the dorsal margins (*dm*). The only passage to the mouth from this entire region is through the notch (indicated by the bent arrow) and when in scores of trials the dorsal tracts were spread apart as far as possible, no large amount of material was ever allowed to pass through it but was always seized by these outgoing tracts. Always, when large quantities of material were brought to this gateway, along the groove of the appendage, as well as along the lateral oral groove, they were halted and turned on to outgoing tracts, especially those of the dorsal margins (*dm*). The dorsal margin of the posterior palp is very wide, and is bent into a broad, shallow trough which is continuous with the groove (*lg*) above it. From the notch *x* there are also outgoing tracts on the sides of the groove of the appendage, as shown in figures 70 and 71, which carry away a small amount of material. Very narrow streams of material, on the other hand, were able to enter between the inner palp faces, and were often found collected there in considerable quantity. It seems as if Drew must have

assumed, and not actually observed, that these extensive appendage collections passed to, and entered, the mouth. Yet it may be said that if they do not, but are usually cast away, it is hard to understand why they are made at all. Nevertheless, in very many observations, extensive collections by the appendages were invariably conducted out of the body on outgoing tracts.

It has been shown that large accumulations, brought to the gateway *x* by appendage or oral groove, are taken up by the dorsal margin tracts and cast into the mantle chamber from the palp tips. There is still another and very remarkable outgoing tract that may be brought into operation at this point and throughout the groove (*lg*) shown in figure 70. The anterior wall of this groove is thick, rounded, and capable of no movement. Collections are brought over it from the outer surface of the anterior or outer palp. The posterior lip of the groove, continuing out on the dorsal margin, is very thin and flexible, and on its outer face (to the left of reference letters, *lg*) is a strong cilia current directed upward. Its course is easily followed, and its loads are seen to be transferred to the inner face of the suspending membrane of the gill (above reference letters *gm* in figs. 68 and 70). Here this waste matter from the palp passes into the epibranchial chamber of the gills, and leaves the body through the excurrent siphon. This flexible groove margin receives its material chiefly from the groove, by rolling over into it in response to the stimulus of a large amount of material, a process similar to the cleaning action of the ventral margins of such palps as those of *Schizothorus* or *Mytilus*, where, by an upward folding, or a spiral twisting of the entire organ, they are brought in contact with the folds.

Palp folds. The palp folds are numerous, and are continued forward very much nearer to the mouth than is usual (as shown in the view of the mouth region represented in fig. 72) so that the proximal oral groove (*pg*) is very short. It is noteworthy that the palp folds nearest the mouth are the smallest, and that they become very large near the palp tip (fig. 70), the reverse usually being the case. The chief current is directly across the folds toward the mouth, while in the grooves, and

apparently always exposed, are narrow, dorsally directed tracts which tend to work material upward to the extremely narrow lateral oral groove (*lg*, fig. 72). No ventrally directed tracts were seen.

Mouth region. Figure 72 represents a very interesting ciliation. Here the anterior palps (*ap*) have been folded forward, and the posterior (*pp*) backward. Instead of forming continuous lips in front of and behind the mouth (*m*) as is usually the case, there is nearly a complete break on the median line, the ventral edge of the anterior fusing with the mantle, which here stretches across between mouth and anterior adductor, about midway between letters *m* and *mo*. The ventral margins of the posterior palps, on the other hand, independently enter the mouth itself, and extend for some distance into its cavity.

In most lamellibranchs, material may approach the mouth only along the very narrow line of the oral groove (*lg* and *pg*) for near the mouth this is bounded by outgoing tracts; but here it may enter all along the sides of the opening. But even after actually entering, it may still be caught up by outgoing tracts, and ultimately be discharged from the body, and this invariably happens in experiment, when more than a very small volume of material attempts to enter at a time. This is repeated again and again on tracts leading to the mouth in nearly all lamellibranchs. If material is to enter the digestive tract, it must be conducted to it in narrow streams, and a little at a time.

The outgoing streams from the mouth opening are three in number. One is on the median line anteriorly, and leads, right and left, to the mantle wall. Another, corresponding to it, is a broad tract from the posterior mouth edge, and leads out on to the visceral mass. The third is found on the ventral margins of the posterior palps, and extends into the mouth itself. Here, however, the outgoing streams appear not to be exposed directly to the mouth cavity, but are on those sides of the palp edges which are next to the wall. They can be exposed, and have been seen to bear out material that had fairly entered the mouth.

Mantle. There is a general ciliation of mantle walls, with no distinct tracts, collections being carried ventralward and back-

ward, and leaving the mantle chamber at the point, just below the siphon, where the palp tentacles are extruded.

SUMMARY

Numerous anatomical features, not previously noticed, are shown in the figures.

Unique organs in the group of the lamellibranchs, are the immense vascular fold arising from the visceral mass of *Schizotherus* (fig. 1), and the collecting membrane of *Barnea costata* and *B. pacifica*, also arising from the visceral mass (figs. 57 and 60). The former is connected with the palps, the latter confined to the posterior wall of the visceral mass, and they are probably not homologous structures. The direction of cilia currents is quite different in the two cases. The organ in *Barnea* is developed to aid and perfect the outgoing cilia mechanism, and apparently can have no other function. The fold of *Schizotherus* is an immense blood reservoir, and its cilia to some degree aid the outgoing system.

The direction of the beat of cilia is never changed.

Cilia streams on the surfaces of organs exposed to the water are divided into two systems, namely, those leading to the mouth, and those bearing material outside the body. The operation of each, in *Schizotherus*, is described in detail.

The numerous currents of all gills belong to the ingoing system, except, among the forms examined, *Yoldia*, *Monia* and *Pecten*, in which the gills possess certain outgoing tracts which are entirely different each from the others. Each of these ciliations is complex and extremely efficient.

The palps, which have attained their present size, form, and position on account of the function of their cilia, are shown to exercise general control over the two ciliary systems, determining whether or not collected material shall enter the digestive tract. The cilia tracts of the palps are shown to have greater complexity and to be more important than those of other organs, and this account deals most fully with their positions and activities. Practically nothing has heretofore been published con-

cerning them. Streams on outer palp surfaces usually pass over the dorsal margins to the inner or applied faces. Those on the inner faces vary greatly in different genera, as will be seen by a comparison of the various figures.

In several forms, parts of the visceral mass collections are passed to the palps. In all cases, the greater part, if not all, is cast into the mantle chamber.

Though there is greater variation in the direction taken by streams on the mantle tracts than on other organs, all belong to the outgoing system, except in a few cases in which small amounts are passed on to the anterior palps.

There is no selection or separation of food organisms from other water-borne particles.

Volume alone determines whether the collected foreign matter that reaches the palps, shall proceed to the mouth, or shall be sent from the body on outgoing tracts. The gills of *Yoldia* and *Pecten* also have the power—by two entirely different mechanisms—of directing their collections on to outgoing tracts when the volume of these is sufficient.

A lamellibranch is able to feed only when waters are comparatively clear—when diatoms are brought to the gill surfaces a few at a time. In muddy waters, all suspended particles, of whatever nature, are led to outgoing tracts. An exception is found in the sand-eating genus *Macoma*.

All ciliated surfaces produce mucus, which appears locally in response to the stimulus afforded by the touch of foreign particles. Its amount is always that necessary to entangle the stimulating particles. Long continued stimulation of any surface may cause relatively enormous quantities of the secretion to be discharged.

In several genera, the mantle develops, on each side, folds lying parallel with the edges, posteriorly (*Schizotherus* and others). These may enclose a waste canal where waste matter accumulates, until carried out of the incurrent siphon or siphonal opening, by a sudden reversal of its stream. A waste canal is made necessary by the presence of a siphon membrane which under certain conditions throws the entering water stream on to

the ventral wall of the mantle space, as described for several forms.

There is much difference in individuals of the same species in the strength or activity of any tract, even where external conditions apparently have been the same. No marked variations in the positions of tracts were noticed.

The aid of the nervous system and of muscles is frequently necessary to the operation of cilia tracts, as in the exposure of hidden outgoing tracts by the spreading apart of palp folds, the apposition of ventral margin and folds, of palps and gills, gills and mantle, and gills and visceral mass. Besides this there are violent expelling contortions of gills, palps, and mantle edge, sudden contractions of adductor muscles, and other similar movements.

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THE MORPHOLOGY OF THE FRONTAL APPENDAGE OF THE MALE IN THE PHYLLOPOD CRUSTACEAN *THAMNOCEPHALUS PLATYURUS* PACKARD

ARTHUR T. EVANS

NINE FIGURES

INTRODUCTION

In 1874 Dr. L. Watson collected near Ellis, Kansas, a singular Phyllopod with a peculiar frontal appendage on the head of the male, belonging to the family Branchiopodidae. These and other specimens collected by Doctor Watson were forwarded to Prof. A. S. Packard, who described ('77) this particular species as *Thamnocephalus platyurus*. Since that date only two collections of the species have been recorded, one by Cockerell in 1912 from Montclair, Colorado, and one by Pearse in 1912 from La Junta, Colorado. The material studied by the writer was obtained by Dr. Max M. Ellis at St. Vrain, Colorado, on May 31, 1912.

Thamnocephalus platyurus is an inhabitant of the stagnant plains-pools of western Kansas and eastern Colorado. These pools are usually very muddy and distinctly alkaline, except immediately after a heavy rain. Since the pools are not permanent and are formed entirely by the collection of surface water, they are subject to a rapid concentration by evaporation. They appear in early spring, dry up during the summer, and again appear with the rains of autumn. The specimens taken at Montclair were found in a pool about 15 feet in diameter, the water area of which had been reduced to about 9 feet by evaporation. This pool varied from a few inches to about 8 feet in depth. About the shore was rubbish and various plains plants, as well as an incrustation of alkali. The specimens studied by the writer were collected in a pool filling the bottom of a 'draw' about 40 feet long and 3 or 4 feet deep near its center. The

water was very muddy and a broad incrustation of alkali extended back several feet from the shore of the pool. In this pool the animals frequented the deeper parts, even the bottom. Occasionally they were seen gliding through the water at a depth of 2 or 3 inches where they were easily observed. *Thamnocephalus* swims like *Branchipus*, with the dorsal surface of the body down, the head acting as the prow of a boat, the long thoracic appendages stroking regularly and rapidly. The vertical position in the water was easily changed by the elevation or depression of the broad, flat tail. Associated with *Thamnocephalus* in this habitat were found *Amblystoma tigrinum*, *Bufo cognatus*, tadpoles, snails, dipterous larvae of several kinds and beetle larvae; as well as large numbers of Phyllopods of the genera *Apus*, *Streptocephalus* and *Estheria*.

Thamnocephalus platyurus is very similar in size and shape to *Branchipus*, except for the peculiar frontal appendage of the male and the broad, depressed, fin-like tail present in both sexes. The end of the tail is not forked, as in many of the other Phyllopods like *Streptocephalus texanus*, but is evenly rounded except for a slight but distinct notch in the extreme posterior part. The thickened posterior end of the abdomen occupies the central third of the tail, while the outer, lateral, portions, which comprise the rest of the tail, are thin and membranous. Each second antenna of the male is composed of a short, thick, fleshy portion, which forms the basal joint; and an outer, recurved, distal portion, composed of a more or less horny tissue. This outer distal joint is quite rigid, and when not in use, it is carried like a recurved tusk, backward under the body. All traces of an inner branch of the second antenna are lacking. In the female the second pair of antennae are long and oar-shaped, and near the distal end broaden laterally, rather abruptly, just before the terminal point is reached. When not in use, the female carries these second antennae folded back under the head and thorax.

In color these animals vary from a transparent to a milky white, specimens preserved in alcohol becoming more or less flesh-colored. The intestine is visible through the middle of the rather thick body, as a dark brown tube colored by the con-

tained food material. The tail of the female bears a large, crescentic spot on each side of the alimentary canal. These spots, which vary from flame scarlet along their posterior margin to light orange yellow (Ridgway's 'color standards,' plates 2 and 3) anteriorly, cover from one-half to three-fourths of the posterior lobe of the tail. Similar spots of a smaller size and a lighter color occur on the tail of the male. The eighteen specimens used in this study varied in length from 15 to 38 mm.

FRONTAL APPENDAGE

Macroscopic structure

The frontal appendage, which is present only in the male, projects in the middle line from the front of the head, directly between the bases of the second antennae. It is from one-half to three-fourths as long as the body proper, and at its base is slightly larger in diameter than the basal joint of the second antenna. The basal one-third of the frontal appendage consists of a single trunk, which then divides into two branches, each of which further subdivides into two parts about midway between their junction with the main trunk and their tips (fig. 1). These secondary branches may or may not be further subdivided, the amount of subdivision and the resulting number of tips depending upon the maturity of the individual. In a male 38 mm. long, the outer of the two secondary branches bore four subbranches, of which the two proximal ones were forked; and the inner branch, three. Just below the division of each of the two large branches a small spur occurs on the inner surface of each branch (fig. 1, *D*). This arrangement of the different branches of the frontal appendage gives the whole a somewhat arch-like appearance when the appendage is correctly spread, since the inner branches leave the main branches at an angle and are directed upward and forward. In the water the animal opens the appendage suddenly and, when seen from above, it appears to be more or less flattened. When not in use or extended, the frontal appendage is closely folded under the anterior end of the body between the bases of the antennae, the smaller tips

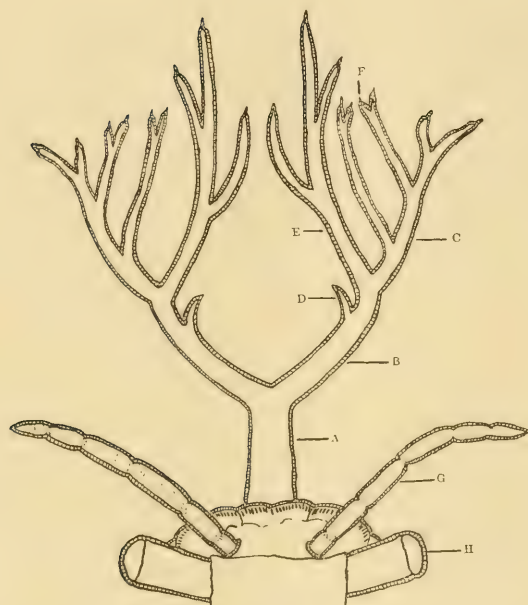


Fig. 1 Dorsal view of frontal appendage and top of head of *Thamnocephalus platyurus*; *A*, trunk of the frontal appendage; *B*, main branch of the trunk; *C*, upper portion of the main branch with its mesial sub-branches; *D*, inner spur of the main trunk; *E*, Principal branch of the main trunk; this branch is directed mesio-dorsally, and with the corresponding branch from the opposite side, forms the dorsal arched portion of the appendage; *F*, forked tips of the mesial branches of the outer portion of the main branch; *G*, first antenna; *H*, eye.

being more or less coiled and the main trunk closed up much like the blade of a jack-knife. The appendage is covered with a thin, flexible layer of chitin resembling a transparent membrane. The tip of each little branch bears a claw-like structure, formed by the rather sudden narrowing of the internal tissues, this narrowed portion tapering to a rather sharp point, the chitinous covering following its contour closely. The whole appendage is more or less roughened by numerous, small, conical projections over its surface. Examination of a number of the smaller and probably immature males showed that the number of branches of the principal branches varied greatly, but they are quite constant in the larger specimens, which would seem to indicate

that the smaller subdivisions of the appendage are added as the animal becomes larger (table 1). In both the young and old specimens it was found that the appendage was slightly flattened on the ventral side, which may be due, however to its contact with other parts while it is closed up under the head.

TABLE 1

Showing the variation in the number of terminal branches and the apparent correlation of number with size, that is, age of the animal

NO. OF SPECIMEN	BODY LENGTH IN MM.	ARRANGEMENT OF BRANCHES ON C ¹			ARRANGEMENT OF BRANCHES ON E ¹	
		No. de- veloped	No. de- veloping	No. forked	No. de- veloped	No. de- veloping
1.....	15	2	1	0	2	1
2.....	20	2	1	1	3	0
3.....	21	3	1	2	3	0
4.....	24	3	1	2	3	0
5.....	38	4	1	2	3	0

¹ See figure 1.

Microscopic structure

For the study of the minute anatomy of the frontal appendage a complete series of cross-sections, beginning with the outer tip and continuing well back into the body of the animal were made in celloidin. This series was used primarily for the reconstruction work. Thin sections of various parts of the head and frontal appendage were made in paraffin. These were stained with iron hematoxylin or safranin-gentian violet. Thick sections were stained with eosin or borax carmine.

A typical cross-section (fig. 2) of a frontal appendage shows the trunk to be composed of several definite areas. A thin, flexible, transparent, chitinous covering surrounds the whole; just inside of this is a layer of epithelial cells, which, although columnar for the most part, are modified in certain regions. In the mid-dorsal and the mid-ventral regions the columnar epithelium is produced vertically so that it joins the mass on the opposite side to form a continuous dissepiment, dividing the appendage into right and left halves. These lateral halves are



Fig. 2 Cross-section of main trunk of frontal appendage of *Thamnocephalus platyurus*; *A*, mid-dorsal mass of modified columnar epithelium; *B*, lateral unmodified epithelium; *C*, area of areolar, adipose, and connective tissues; *D*, lateral area of modified columnar epithelium, forming lateral insertion for flexor muscle; *E*, flexor muscle; *F*, ventral sinus; *G*, ventral mass of modified columnar epithelium; *H*, mesial junction of the dorsal and ventral masses of modified columnar epithelium; the dissepiment thus formed is the inner insertion of both flexor muscles; *I*, chitinous covering of the appendage.

quite similar, each containing in its dorsal portion a considerable amount of adipose and connective tissue; and in its ventral portion a large flexor muscle and a conspicuous crescentic sinus. This sinus is located just inside of the ventral layer of epithelium, in the angle formed by the attachment of the inner edge of the flexor muscle to the central dissepiment of modified columnar epithelium.

The columnar epithelial cells occupying the mid-dorsal and mid-ventral regions and the extreme lateral portions of the cross-section—that is, the points of attachment of the flexor muscles—are of a modified type. They contain large numbers of very minute fibrils in the cytoplasm, which give them strength for the attachment of muscles. This particular form of modified

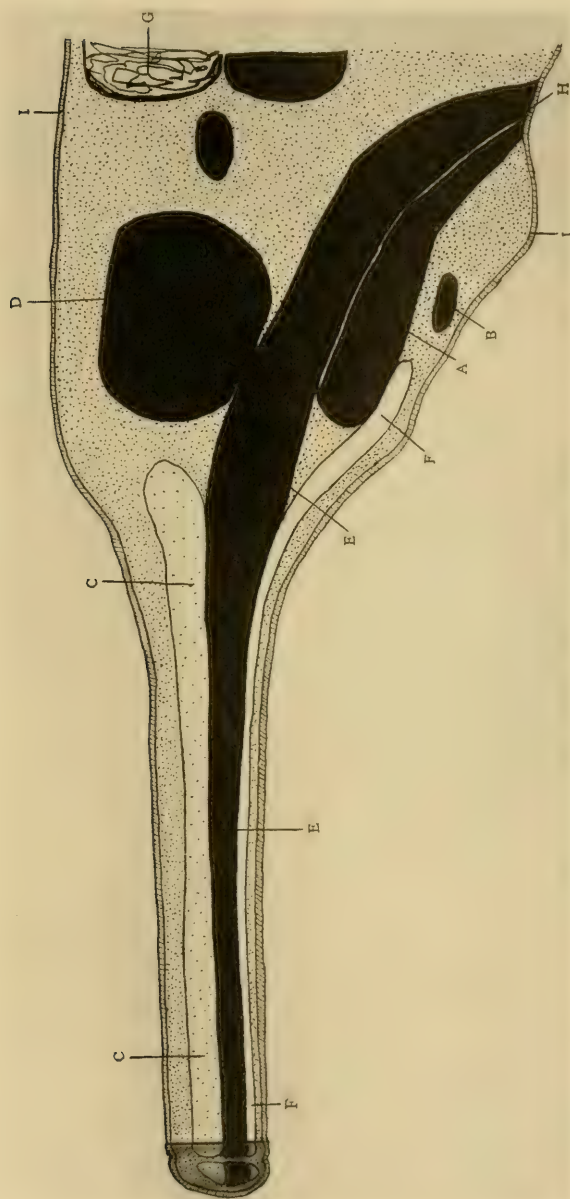


Fig. 3 Stereographic reconstruction of the head and trunk of the frontal appendage of *Thamnocephalus*, side view; *A*, muscle of second antenna; *B*, lateral bundle of the muscle of the frontal appendage; *C*, dorsal mass of areolar, adipose, and connective tissues in the frontal appendage; *D*, a transverse muscle of head; *E*, flexor muscle of frontal appendage; *F*, ventral sinus of frontal appendage; *G*, supra-oesophageal ganglion; *H*, origins of flexor muscle of frontal appendage and the muscle of second antenna; *I*, chitinous covering of appendage.

columnar epithelium has been described by Dahlgren and Kepner ('08, p. 65) who state that in the lobster "the simple columnar epithelium cells that cover the outside of the body under the shell, which they form, assume part of this duty [that of a short ligament] and acquire strength to perform it by the development of strong fibrils in their cytoplasm." The central dissepiment cells flares out at the dorsal and ventral ends, where it comes into contact with the chitinous covering, and all its cells contain fibrils. At the lateral points of attachment of the flexor muscle a wing-shaped expanse of cells, which extends toward the point of attachment of the muscle, shows well developed fibrils in the cytoplasm of the cells. All other epithelial cells are of the columnar type, except midway between the dorsal and lateral portions, where there is a small area of cuboidal epithelium.

Beginning with the dorsal portion of the central dissepiment and proceeding around either side of the appendage, the layer of epithelium changes gradually from modified columnar to simple columnar, then to cuboidal, again to columnar and finally to modified columnar epithelium in the extreme lateral region at the point of attachment of the flexor muscle. This same sequence of changes in the character of the epithelium may be noted if the layer of epithelial cells be followed from the lateral attachment of the flexor muscle to the median insertion of the ventral portion of the central dissepiment of modified columnar epithelium.

The adipose and connective tissues are loosely arranged in the upper portion of the appendage, producing an areolar mass.

The large flexor muscle has for its inner point of attachment a position about midway between the median dorsal and ventral ends of the dissepiment. Its outer point of attachment is the lateral mass of modified columnar epithelium containing fibrils, as well as some of the columnar epithelium which lies along the ventral surface of the appendage.

The large sinuses occupying a ventral median position supply the appendage with blood.

No nerve fibers or sensory cells were found in the appendage, but no special neurological stains were used.

In tracing the various parts of the large trunk to their endings in the tips of the branches, cross, as well as longitudinal, sections were used. At the forking of the large trunk, the right and left halves which compose it separate, one part going to each branch. In these branches as well as the various sub-branches the different tissues occupy the same relative positions as they do in either half of the trunk. All the tissues extend into the very tips of the appendages and in all the branches the flexor muscle occupies an area proportional to that taken by it in the half of the main trunk from which it is derived, the muscle tapering gradually from the main trunk to the tips of the various branches. The parts of the diminishing muscle find attachment on the lateral and ventral walls of the particular branch which they occupy.

Posteriorly, the large flexor muscle increases in size, reaching its greatest thickness just after its entry into the head. At this point it bends slightly ventro-laterally and is met by the large muscle of the second antenna. From this point these two muscles bend still farther in the same direction, and at the same time flare out into a fan-shaped bundle, extending to their point of origin on an inbending of the chitinous covering just in front of the large mandibles (fig. 3). In passing through the head to their point of attachment these two muscles are in such close proximity that they appear almost as a single large muscle.

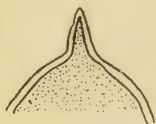


Fig. 4 Optical section of tip of branch of frontal appendage of *Thamnocephalus platyurus*.

The antennal muscle does not lie beside and in the same plane with the retractor muscle of the frontal appendage, but in a more ventro-lateral position along its mesial surface. The muscles in the distal end of the appendage extend out into the small, claw-like structures as small bundles. Whether these claw-like tips (fig. 4) are used in clasping or as tactile organs is unknown.

The large sinus found in each half of the basal trunk, branches with the muscle into the various parts of the appendage, finally ending in the extreme outer parts of the tip in small capillary tubes. After entering the head the sinuses bend toward the median line, pass between the two large retractor muscles from the frontal appendage and join in a single large sinus just back of the esophagus.

The adipose and connective tissue areas in the smaller branches of the appendage are loosely arranged. Next to the muscle the adipose and connective tissues occupy more space in the appendage than any other.

Summary of structures

1. The appendage consists of a thin, chitinous, outer covering; of columnar epithelial cells which may be simple, cuboidal or modified, the latter containing fibrils in their cytoplasm; of a large, longitudinal muscle in the ventral portion of the appendage; of a large, ventral, crescentic sinus; and of areas of areolar, adipose, and connective tissues.

2. The flexor muscle has its origin on a chitinous ridge just in front of the mandibles, and extends along the ventral part of the appendage, divides and subdivides in the various branches where it has its points of insertion on the floor and sides at the outermost points.

3. The sinuses arising in the tips as capillary tubes, extend along the ventral floor of the appendage below the large flexor muscle to a point just within the head, where they bend mesially, passing between the flexor muscles and extending to a point just posterior to the esophagus where the two join in a common sinus.

COMPARISON OF THE FRONTAL APPENDAGE WITH THE INNER BRANCH OF THE SECOND ANTENNA OF OTHER PHYLLOPODS

The second antenna of the Phyllopod, with its various modifications found in the many different species, has been the subject of considerable study; and almost without exception the function of a clasping organ has been assigned to it. In some Phyllopods

the second antenna is composed of a single, large basal joint and two distal, many-jointed branches. *Estheria jonesii* Baird furnishes an example of such a second appendage (fig. 5). Another species which shows a different antenna is *Streptocephalus texanus* Packard (fig. 6) in which one of the branches of the large basal portion is found to be vestigial; the other branch is developed into a gnarled, tortuous, many (usually four or five)

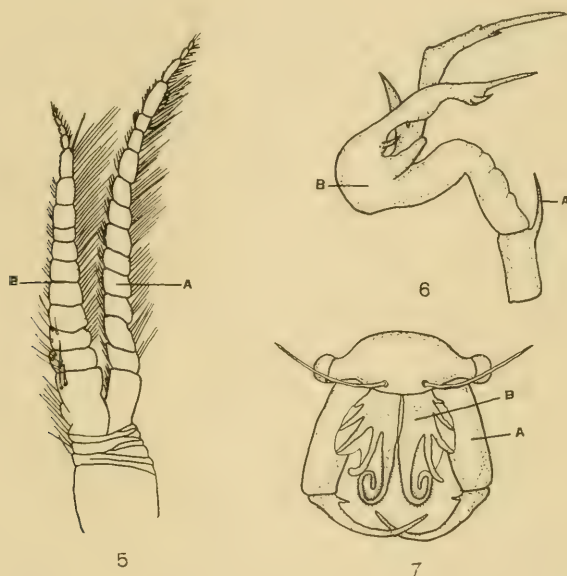


Fig. 5 Second antenna of *Estheria jonesii*. (after Packard); A, ventral branch; B, dorsal branch.

Fig. 6 Second antenna of male *Streptocephalus texanus*; A, unspecialized; B, specialized part.

Fig. 7 Front of head of *Chirocephalus diaphanus*, showing the peculiar second antennae of the male (after Lankester); A, outer branch; B, modified inner branch.

parted appendage, to which the function of a clasping organ has been assigned. In *Chirocephalus diaphanus* (fig. 7) the development seems to have gone a step farther and both branches of the second antennae are intact, but the inner branch is greatly developed, consisting of a large main branch which is divided into several smaller parts. Near its point of attachment to the basal portion of the antenna proper a large fan-like division arises

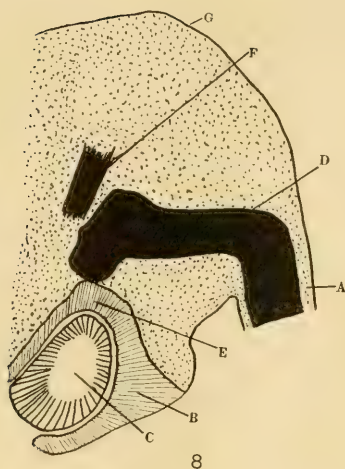
from it. As yet no use has been assigned to this inner portion, although it probably functions with the other parts of the second antenna during copulation. The outer portion of the antenna consists of a long, hard, horny, sickle-shaped hook which, when not in use, is bent back under the body. The inner portion of the appendage is not attached to the distal end of the basal portion, but more nearly to the proximal end. That the inner portion is not sensory is assumed by Packard ('78, p. 351) because no nerves had been found in it. In the female, however, Lankester ('09, p. 36) has assigned the probable function of a sensory organ to the second antenna. The muscular structure of the second antenna, which is mentioned by Packard, probably marks it as an appendage of some importance. Whether the inner branch functions in connection with the other parts of the second antenna as a clasping appendage or as an organ for stroking the female during the process of copulation is a point which can hardly be decided without witnessing the animals in coitu.

In *Thamnocephalus platyurus* the peculiar inner part of the second antenna is lacking but in its stead is the single large frontal appendage. Although this appendage outwardly shows no sign of being double, a cross-section of it, as has been pointed out before, shows that it has probably been formed by the union of the two separate parts. From the variation found in the second antenna of Phyllopods, ranging from the nearly symmetrical appendage in *Estheria*, through the different forms to *Chirocephalus* (in which the inner part has migrated to a position near the proximal end of the basal segment of the antenna proper), it seems quite logical to assume that this development has gone still farther in *Thamnocephalus* and that the inner parts of the second antennae have not only separated entirely from the antenna proper, but that the two parts have met in the median line of the head and there completely fused. This seems all the more logical when it is known that the inner branches of the antennae are wanting. It might be assumed that the inner branches of the antennae were vestigial and that their degeneration had gone so far as to entirely eliminate them in the adult. Such an

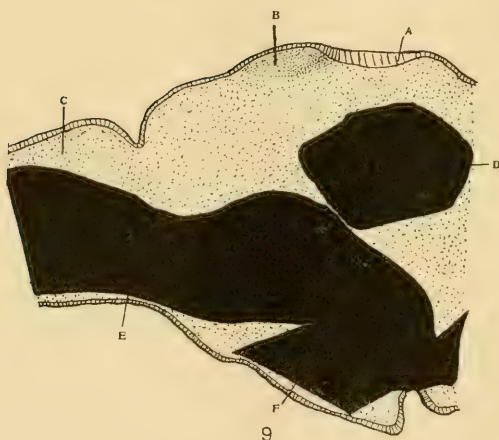
assumption would seem to be entirely overshadowed by the presence of this singular, double, frontal appendage which may be accounted for by the explanation of the fusion of the inner branches of the second antennae. If the two parts of the second antennae in *Chirocephalus diaphanus*, which are known as the frontal appendage, can be conceived as having moved into the mid-dorsal surface of the head and there united at their bases, the branching of the appendage thus formed would not differ greatly from that of *Thamnocephalus*. Another argument which adds weight to the belief that the frontal appendage is of the same morphological origin as the inner branch of the second antenna of some of the species of Phyllopods is the fact that the muscles of the antennae and those of the frontal appendage extend through the head for some distance together, appearing almost as a single muscle on each side, and finally finding their insertions at the same points (fig. 3). The antennal muscles of *Streptocephalus texanus* are attached in the same relative position as those of *Thamnocephalus platyurus* (fig. 8).

On first sight it might seem probable that the queer appendage in *Thamnocephalus platyurus* may have formed by a development of the frontal organ of Claus, which is located in the same general region, but that this is not the case may be seen from the camera lucida drawing of a sagittal section through the parts under consideration (fig. 9), in which it will be seen that the frontal organ of Claus is located several millimeters posterior to the point of junction of the frontal appendage with the head, and that it has the same general structure as the organ of Claus in *Chirocephalus*.

The function of the peculiar frontal appendage of *Thamnocephalus* is not known, but, with a knowledge of its morphology, some assumptions as to its use may be made. In life the animal usually keeps the appendage coiled up closely under the head. Now and then, however, it is extended in front of the animal in a very conspicuous fashion. There are no muscles for expanding the appendage, so that it must be pushed out in some other way, probably by the distension and subsequent inflation of the large sinuses with blood. Upon filling, the sinuses would expand the



8



9

Fig. 8 Sagittal section through head of *Streptocephalus texanus*, showing origin of flexor muscle of the second antenna; *A*, base of second antenna; *B*, ventral thickening of chitinous covering of body, in front of the mandible; *C*, cross section of base of mandible; *D*, flexor muscle of second antenna; *E*, thickening of chitinous covering of body at origin of flexor muscle; *F*, one of the transverse muscles of head, in section; *G*, chitinous covering of body.

Fig. 9 Para-sagittal section of head and base of frontal appendage of *Thamnocephalus platyurus*, showing frontal organ of Claus; *A*, chitinous covering; *B*, frontal organ of Claus; *C*, base of the trunk of frontal appendage; *D*, a transverse muscle of head; *E*, muscle of frontal appendage; *F*, muscle of second antenna.

appendage. With the contraction of the large flexor muscle the blood is forced backward through the sinuses into the large sinus with which they connect. As the muscle of the appendage is large the structure might be used as a strong clasping appendage. It is hard to understand, however, just how this median structure might thus be used, if copulation (which has not yet been observed in *Thamnocephalus platyurus*) takes place in a way similar to that in which it occurs in *Artemia gracilis* Verrill, as figured by Packard ('78, fig. 17). If copulation does take place in a similar manner and the appendage does not function as an additional clasping organ then it is possible that the appendage is used as an organ for stroking the female on the back while in coitu.

In conclusion, it may be stated that the function of a sensory organ is apparently eliminated by the lack of nerves or sensory cells in the structure. The large muscles would indicate that it is very likely used for seizing and holding, which would put it in the category of a purely copulatory structure.

Whether the function is that of a clasping or stroking organ must remain an open question until the animals are more closely observed in the field and actually seen in the process of copulation.

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